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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

SECTION HEAD

OFFICE OF PESTICIDE PROGRAMS
Health Effects Division

OCT - 3 1995

MEMORANDUM

SUBJECT: Benoxacor: Review of a Chronic Oral Toxicity study in Dogs (§83-1), a Carcinogenicity Study in Mice (§83-2), a Combined Chronic Toxicity/Carcinogenicity Study in Rats (§83-5), and a Reproductive Toxicity Study in Rats (§83-4)

TO: Mary Waller - Acting PM 45
Registration Support Branch, Registration Division (7505W)

FROM: David S. Liem, Ph.D. David Liem 9/28/95
Section II, Toxicology Branch II, HED (7509C)

THROUGH: Clark Swentzel, Section Head K. Clark Swentzel 9/29/95
Section II, Toxicology Branch II, HED (7509C)
and
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Acting Branch Chief, Toxicology Branch II, HED (7509C)

Barcode: D194579; Submission#: S446951; ID#: 7E03489; PC#: 126101
MRID#: 428887-01, -02, -03, and -04

Chemicals: CGA 154281; Benoxacor
Synonyms: 4-dichloroacetyl-3,4-dihydro-3-methyl-2H-1,4-benzoxazine (CAS# 98730-04-2).

Action Requested: Review a Chronic Oral Toxicity study in Dogs (§83-1), a Carcinogenicity Study in Mice (§83-2), a Combined Chronic Toxicity/Carcinogenicity Study in Rats (§83-5), and a Reproductive Toxicity Study in Rats (§83-4) using benoxacor submitted by Ciba Geigy Ltd of Switzerland. Benoxacor (CGA 154281) is to be used as a safener.

Executive Summary: Results of the evaluations of the toxicity studies are summarized below:

1. 52-Week Oral (Capsule) Toxicity Study in the Beagle. J.D. Wood, September 7, 1992. Study#6904-380/158 (MRID#428887-01).

Benoxacor (purity 96.8%) was administered orally in capsules to male and female beagle dogs (4/sex/group) at doses of 0, 1, 5, 40 and 80 mg/kg/day for 52 weeks. At 80 mg/kg/day, a slight increase in incidence and severity of pigmentation in the proximal tubule of the kidney in males was observed. In addition, at the 80 mg/kg/day dose level there was a slight indication of hemolytic

anemia, expressed by decreased red cell parameters (erythrocyte count, hematocrit, hemoglobin, mean cell volume and mean cell hemoglobin) in males and increased bilirubin in males. At 40 mg/kg/day dose level, decreases in mean body weight gain in males, increases in adjusted liver and kidney weights and increased lipofuscin deposition in the kidneys in both sexes, were also noted.

The systemic toxicity NOEL is determined to be 5 mg/kg/day. The systemic toxicity LOEL is 40 mg/kg/day based in decreases in mean body weight gain in males, increases (both sexes) in adjusted liver and kidney weights, and increased lipofuscin deposition in the kidneys of both sexes.

This study is classified as core guideline and it satisfies the guideline requirements (§83-1) for a chronic oral toxicity study in dogs.

2. Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice. Peter R. Ryle. May 5, 1993. Study# CBG 510/911356 (MRID#428887-02).

Benoxacor (CGA 154'281; 96.4% pure) was fed to male and female CD-1 mice (50/sex/group) at dietary levels of 0, 10, 30, 600 and 1200 ppm for 80 weeks. The average daily intakes were 1.2, 3.7, 75 and 167 mg/kg/day for males and 1.6, 4.7, 93 and 201 mg/kg/day for females.
The following treatment-related effects were observed:

- Body weight decreased in the 1200 ppm males
- Increased liver/body weight ratio in 600 ppm and 1200 ppm in both males and females.
- Increased forestomach excrescences and thickening of limiting ridge in 600 ppm and 1200 ppm in both males and females.
- Decreased adipose tissue in 1200 ppm males
- Increased incidence of pale kidneys in 1200 ppm males
- Increased liver enlargement in 1200 ppm females
- Increased incidence of papillomatous hyperplasia of the stomach and epithelial hyperplasia of the forestomach in 1200 ppm males.
- Increased spleen hemosiderosis, hemorrhagic ovarian cysts and parenchymal inflammatory hepatic cells were in 1200 ppm females.
- Squamous cell papilloma of the stomach in 1200 ppm males and females

Based on the above data the systemic toxicity NOEL is determined to be 30 ppm (3.7 and 4.7 mg/kg/day in males and females, respectively). The systemic toxicity LOEL is 600 ppm (75 and 93 mg/kg/day in males and females, respectively), based on increased signs of stomach toxicity consisting of forestomach excrescence, and increased liver/body weight ratio in both sexes.

This study is classified as core guideline and it satisfies the guideline requirements (§83-2) for a carcinogenicity study in mice.

3. CGA 154'281: Combined Chronic Toxicity/Oncogenicity study in Rats. Potential Tumorigenic and Toxic Effects in Prolonged Dietary Administration to Rats. Peter R. Ryle. April 27, 1993. Study# CBG 509/9205599 (MRID#428887-04).

Benoxacor (CGA 154281; 96.3% pure) was fed to male and female Crl: CD BR rats (50/sex/group) at dietary levels of 0, 10, 50, 500 and 1000 ppm (0, 0.4, 2.0, 20.6 and 41.0 mg/kg/day in males and 0, 0.6, 2.8, 28.2 and 59.0 mg/kg/day in females) for 2 years.

Treatment-related body weights and body weight gains, food consumption and feed efficiency were decreased in males and females at 500 ppm (20.6 mg/kg/day for males and 28.2 mg/kg/day for females) and 1000 ppm (41 mg/kg/day for males and 59 mg/kg/day for females).

Treatment-related decrease of total protein, globulin and adipose tissue were noted in the 1000 ppm males. Liver/body weight ratio was increased in the 1000 ppm males.

At 10 ppm (0.4 mg/kg/day in males and 0.6 mg/kg/day in females), increases in the incidence of fatty hepatocytes, parafollicular cell hyperplasia in the thyroid and basophilic cortical tubules in the kidneys were observed in males; in addition, 10 ppm females had congested kidneys and epithelial hyperplasia and hyperkeratosis of the forestomach.

Histological lesions at higher doses included liver effects such as cystic bile duct in 1000 ppm females and centrilobular hypertrophy (with or without vacuolation) in the 50, 500 and 1000 ppm males; changes in heart pathology were noted in the 500 and 1000 ppm males; stomach lesions such as raised areas on the epithelial aspect of the forestomach were noted in 1000 ppm males and females, nodular and papillomatous hyperplasia of the limiting ridge were noted in 500 and 1000 ppm males as well as excrescence and papillomatous hyperplasia of the nonglandular stomach in 1000 ppm females. An increased incidence of ovaries without corpora lutea and with follicular cysts were noted in 1000 ppm dose females

Peto analysis revealed statistically significant trends for increases in the incidences of squamous cell papillomas in males and squamous cell carcinomas (and carcinomas and/or papillomas) in females in the stomach (epithelial portion of the nonglandular region and/or limiting ridge) at doses tested under the conditions of this study. However, the incidences of these tumors were not significantly increased by pairwise comparison at any dose.

Based on the above data, the systemic toxicity NOEL is determined to be <u>less</u> <u>than</u> 10 ppm (<0.4 in males and <0.6 mg/kg/day in females, respectively). The systemic toxicity LOEL is 10 ppm (0.4 mg/kg/day in males and 0.6 mg/kg/day in females), based on significant increases in the incidences of fatty hepatocytes in males, parafollicular cell hyperplasia in the thyroids of males, basophilic cortical tubules in male kidneys and congestion in female kidneys.

CORE CLASSIFICATION: The chronic study is classified as core-supplementary because the systemic NOEL could not be determined. However, the carcinogenicity study is classified as core-minimum and satisfies the guideline requirements (§83-2) for a carcinogenicity study in rats.

4. Two-Generation Oral (Dietary Administration) Reproduction Toxicity Study in the Rat on CGA 154281 (One Litter per Generation). I. Osterburg. December 1991. Study# 380-154 (MRID#428887-03).

In a two-generation reproduction study, Sprague-Dawley rats received benoxacor (96.8% pure) continuously in the diet for two successive generations at dosages of 0, 10, 50, 500 and 1000 ppm. In the P generation, the mean compound intake during premating was 0.69, 3.55, 34.84 and 68.80 mg/kg/day for males and 0.81, 4.51, 41.21 and 82.31 mg/kg/day for females. For P females during gestation, the mean compound intake was 0.72, 3.56, 35.27 and 70.66 mg/kg/day and during lactation, it was 1.36, 6.53, 64.02 and 133.51 mg/kg/day.
During premating, the mean compound intake for F1 males was 0.83, 4.20, 45.45, and 89.21 mg/kg/day and for F1 females was 0.92, 4.57, 49.16, and 93.53 mg/kg/day. For F1 females during gestation, the mean compound intake was 0.73, 3.67, 37.65 and 73.65 mg/kg/day and during lactation, it was 1.5, 7.47, 73.25 and 149.31 mg/kg/day.

At 500 ppm and 1000 ppm, treatment-related decreased body weight and body weight gains in both sexes of both P and F1 generations were noted. In addition, a food consumption decreases were noted in the P males during the premating period and in the 1000 ppm F1 males and females during the treatment period. Significant pup body weight decreases were noted at lactation day 21 in F1 pups in 500 ppm and 1000 ppm dose groups and in 1000 ppm F2a pups. On day 14 post-partum the 1000 ppm F1a pups' weight was also reduced.

Banoxacor did not induce detectable changes on the fertility or the reproductive performance, the reproductive organs or in any organs in P and F1 animals dosed up to 1000 ppm. No treatment-related malformations of the F1 and F2a pups were observed.

Administration of Benoxacor at 10 and 50 ppm, did not produce any treatment-related toxic effects on P and F1 rats or their pups.

Based on the data as presented in the study report, the parental NOEL is determined to be 50 ppm (3.55 mg/kg/day in males and 4.51 mg/kg/day in females) and the parental LOEL is 500 ppm (34.84 mg/kg/day in males and 41.21 mg/kg/day in females) based on decreased body weight and body weight gain in both sexes and in both generations.
The reproductive NOEL is determined to be 50 ppm and the reproductive LOEL is 500 ppm based on decreased pup body weight on lactation day 21 in both F_1 and F_2 generations.

This study is classified as core guideline and it satisfies the guideline requirements (§83-4) for a reproduction study in rats.

GENERAL CONCLUSIONS: **Please note that the conclusion on Benoxacor can not be made at this time, because the Registrant has yet to submit a Forward Mutation Study. Furthermore, since there is evidence of treatment-related neoplastic lesions in both the rat and mouse studies that have been evaluated (see above summaries and the attached DERs), these studies should be reviewed by the HED Cancer Peer Review Committee, before the Toxicology Branch II can consider the requested petition in support of a tolerance exemption for Benaxacor as a safener under 40CFR180.1001.**

Benoxacor (syn.= 4-dichloroacetyl-3,4-dihydro-3-methyl-2H-1,4-benzoxazine; CAS# 98730-04-2) is not listed in the USEPA's TRI list.

Benoxacor DER 10/3/95

Page ___ is not included in this copy.

Pages 6 through 21 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Primary Reviewer:, David S. Liem, Ph.D. David Liem 9/15/95
Toxicology II, Section II, HED
Secondary Reviewer: Clark Swentzel M. Clark Swentzel 9/15/95
Toxicology Branch II, Section II, HED

DATA EVALUATION REPORT

STUDY TYPE: Carcinogenicity study in mice (GuideLine 83-2)

MRID NO.: 428887-02 DP BARCODE#: D194579 ID#: 7E03489

SUBMISSION#: S446951 PC No.: 26101 CASWELL #: none

TEST MATERIAL: CGA 154281 (Benoxacor)

SYNONYM: 4-dichloroacetyl-3,4-dihydro-3-methyl-2H-1,4-benzoxazine (CAS# 98730-04-2).

DOSE LEVELS: 0, 10, 30, 600 and 1200 ppm (0, 1.2, 3.7, 75 and 167 mg/kg/day for males and 0, 1.6, 4.7, 93 and 201 mg/kg/day for females).

STUDY NO.: CBG 510/911356

SPONSOR: Ciba-Geigy Corporation, Ciba Plant Protection, Greensboro, NC 27419

TESTING FACILITY: Huntingdon Research Centre Ltd.,Huntingdon, Cambridgeshire, England

TITLE OF REPORT: Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice

AUTHOR: Peter R. Ryle REPORT ISSUED: May 5, 1993

QUALITY ASSURANCE: A signed Quality Assurance Statement (dated May 5, 1993) and a signed GLP Certification Statement (dated June 28, 1993) were provided. The study was conducted in compliance with OECD guidelines, GLP regulations, EPA FIFRA pesticide guidelines, and Japanese guidelines.

EXECUTIVE SUMMARY: Benoxacor (CGA 154281; 96.4% pure) was fed to male and female CD-1 mice (50/sex/group) at dietary levels of 0, 10, 30, 600 and 1200 ppm for 80 weeks. The average daily intakes were 1.2, 3.7, 75 and 167 mg/kg/day for males and 1.6, 4.7, 93 and 201 mg/kg/day for females.

The following treatment-related effects were observed:
- o Body weight decreased in the 1200 ppm males
- o Increased liver/body weight ratio in 600 ppm and 1200 ppm in both males and females.
- o Increased forestomach excrescences and thickening of limiting ridge in 600 ppm and 1200 ppm in both males and females.
- o Decreased adipose tissue in 1200 ppm males

- Increased incidence of pale kidneys in 1200 ppm males
- Increased liver enlargement in 1200 ppm females
- Increased incidence of papillomatous hyperplasia of the stomach and epithelial hyperplasia of the forestomach in 1200 ppm males.
- Increased spleen hemosiderosis, hemorrhagic ovarian cysts and parenchymal inflammatory hepatic cells were in 1200 ppm females.
- Squamous cell papilloma of the stomach in 1200 ppm males and females

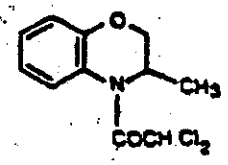
Based on the above data the systemic toxicity NOEL is determined to be 30 ppm (3.7 and 4.7 mg/kg/day in males and females, respectively). The systemic toxicity LOEL is 600 ppm (75 and 93 mg/kg/day in males and females, respectively), based on increased signs of stomach toxicity consisting of forestomach excrescence, and increased liver/body weight ratio in both sexes.

This study is classified as core guideline and it satisfies the guideline requirements (§83-2) for a carcinogenicity study in mice.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: CGA 154281
Structure:



Composition: 4-Dichloroacetyl-3,4-dihydro-3-methyl-2H-1,4-benzoxazine.
Batch number: FL 8816261
Purity: 96.4%
Physical property: Brown crystalline powder
Stability: Stable for at least 3 years (reported by sponsor)
Storage: At room temperature protected from light

2. Test Substance Analyses for Purity and Stability

Test diets containing 96.4% pure CGA 154281 were prepared weekly by weighing out a specified amount of the basal diet, adding the appropriate amount of test material, and grinding in a Turbula mixer for a minimum of 2 minutes. Two premixes were prepared: one for the 1200 ppm diet and one for the 30- pm diet. The 600 and 10 ppm diets were prepared by diluting the appropriate premixes and then reblending. Storage conditions were not

and then reblending. Storage conditions were not described.

Concentration of CGA 154281 in the diets was determined by extraction with dichloromethane and quantitation by HPLC with ultraviolet detection. Homogeneity of the diet was determined before study initiation with nominal concentrations of 10, 20, and 5000 ppm. Samples were taken from the top, bottom, and center of the container. Samples taken from the 10 ppm diet showed a 1.49% coefficient of variance of composition. The 20 ppm and 5000 ppm test diets showed 4.92% and 0.81% coefficients for variance of composition, respectively. Thus, the procedure used to mix the diets was considered adequate, and the diets were considered homogenous. The mean concentration of the samples ranged between 97% and 102% of the intended levels.

Analyses of the stability of CGA 154281 in the diet were performed from subsamples of the prepared 10, 20, and 5000 ppm mixes. The samples were stored under four different conditions: frozen at -20° C, or for 4, 8/10, or 18 days under animal room conditions (not described in detail). Results for the samples stored frozen were not given. CGA 154281 was stable for up to 18 days under animal room conditions with recoveries of 92% to 102% of nominal concentration at day 18.

Analyses of CGA 154281 concentration in the diet were performed at all dietary levels during study weeks 1, 5, 9, 15, 21, 27, 33, 39, 45, 51, 57, 63, 69, 75, and 81. Target concentrations and the means of actual measured concentrations were as follows:

	TARGET CONCENTRATIONS*			
	10 ppm	30 ppm	600 ppm	1200 ppm
Mean Concentration (ppm)	9.91	30.45	576.47	1193.33
Standard Deviation (ppm)	0.39	1.37	15.02	36.58
Percentage of Target	99	101	96	99

a Data extracted from Study No. CBG 510/911356, Addendum 4, (pp. 1616-1618)

3. Animals

A total of 585 Crl:CD-1(ICR)BR mice (291 males and 294 females) were purchased from the Portage, Michigan, branch of Charles River Laboratories, Inc. Upon arrival, 5 males and 5 females were chosen randomly for the health examination. These animals were killed within 24 hours of arrival, and tissues were examined macroscopically; if abnormalities were seen, they were also examined microscopically. Animals were randomly assigned to five groups (50/sex/group) so that no statistical differences in mean body weights existed and were acclimated to laboratory

conditions for 12 days. At the initiation of the study, the animals were approximately 6 weeks of age, and weight ranges were 20-33 g for males and 18-33 g for females. Animals were housed by sex 2 per cage in solid-bottom polypropylene cages that contained autoclaved sifted sawdust. SDS Rat and Mouse No. 1 modified maintenance diet and tap water were provided ad libitum at all times. Food and water were analyzed for environmental contaminants. Caging and sanitary conditions were maintained in accordance with the SOPs of the testing facility. The mice (50/sex/group) were randomly Assigned to the following test and control groups:

Dose Group	Dosage (ppm)	Number of Animals	
Control	0	50 ♂	50 ♀
Low	10	50 ♂	50 ♀
Mid-Low	30	50 ♂	50 ♀
Mid-High	600	50 ♂	50 ♀
High	1200	50 ♂	50 ♀

4. Rationale for Dose Selection

Doses were based on the results from a 13-week range-finding study (Ciba Geigy Project number 891290). The results of this study were discussed in Study No. CBG 510/911356. Doses of 0, 50, 500, 2000, and 6000 ppm were administered daily. Adverse effects on the kidney and the liver and decreased food intake and weight gain were seen at doses of 2000 and 6000 ppm. Slight increases in liver and kidney weights were seen at 500 ppm in both sexes. No details on the evidence for hepatotoxicity and nephrotoxicity were given. The maximum tolerated dose for CGA 154281 was considered to be between 500 and 2000 ppm. Thus, a high-dose of 1200 ppm was chosen for the oncogenicity study.

5. Statistical Analyses

Food consumption, body weight, and organ weight data were analyzed using Bartlett's test for heterogeneity of variance between treatments. If no significant heterogeneity was found, a one-way analysis of variance or covariance (ANOVA or ANCOVA) was used. If significant heterogeneity that could not be removed by logarithmic transformation was found, Kruskal-Wallis analysis of ranks was used. Student's T test and William's test were used to determine dose related responses. Food consumption was analyzed on a per cage basis. Mortality was analyzed using log rank methods. Incidences of tumors were analyzed using Fisher's exact test or by Peto's methods. Shirley's test was also used.

6. General Observations

(a) Mortality/Moribundity/Survival

Animals were observed daily for mortality and moribundity.

Results: No treatment-related effects on mortality were observed. Percent survival at study termination ranged from 64% in the high-dose males to 78% for the mid-high-dose males. In treated females, percent survival at study termination ranged from 72% to 78%. Percent survival rates for the control groups were 76% and 70% for males and females, respectively.

(b) Clinical signs

Observations for adverse clinical effects were made once daily. Physical examination was conducted daily for the first four weeks of the study and weekly thereafter.

Results: No treatment-related clinical signs were observed. No treatment-related differences in the incidence, type, and location of palpable masses were observed.

(c) Body weights/Body weight gains/Food consumption

Body weights/body weight gains: Body weights were recorded once weekly throughout the study.

Results: Mean body weight and mean body weight gain data are summarized in Tables 1 and 2. Mean body weights were consistently and significantly decreased in high-dose males for weeks 7 through 80 in the study (89-97% of control) except during weeks 9, 15, and 17. A slight (97% of control value) but statistically significant decrease in mean body weight was observed in the high-dose females at week 0. Following week 0, mean body weights in the high-dose females were, for the most part, comparable to those of controls. Mean body weight gain was significantly decreased in high-dose males when compared to controls; weeks 0-13, 78% of control value and weeks 0-52, 81% of control value. No treatment-related changes in body weight gain were observed in females.

Food consumption--Food consumption was calculated weekly for each mouse (g/mouse/week).

Results: No treatment-related changes in food consumption were observed. At study termination, high-dose males had consumed slightly more food than controls (102% of control value), although they had significantly decreased body weight gains when compared to controls. Efficiency of food utilization (food consumption [g]/body weight gain (g]) was calculated for the maximal growth intervals (weeks 1-13). Food efficiency was slightly impaired for high-dose males (55.2 compared to 43.1 in controls).

7. Clinical Pathology

Venous blood samples were obtained from all animals that died during the study, when possible, and from all surviving animals during weeks 52 and 78 of the study. Leukocyte differential counts and erythrocyte morphology were examined on blood smears that were placed on slides, fixed in methanol, and stained with Wright's stain. No information regarding method of blood collection was provided.

Results: No statistically significant changes in hematology parameters were observed.

8. Sacrifice and Pathology

All animals that died during the study or were sacrificed either moribund or at study termination (by carbon dioxide asphyxiation) were given a complete postmortem examination. Gross examination was performed both visually and by palpation for distortion, swelling, or other evidence of tumor formation. Tissues were preserved in 10% neutral buffered formalin (except for the eyes which were preserved in Davidson's fixative). Tissues marked with an "X" below were examined histologically in all animals. Organs indicated by "XX" below were also weighed for all animals sacrificed at study termination. * = recommended by Guidelines.

Digestive System	Cardiovascular System	Nervous System
X Tongue	X Aorta*	XX Brain*
X Salivary glands*	XX Heart*	X Peripheral nerve
X Esophagus*	X Bone marrow*	X Sciatic nerve)*
X Stomach*	X Lymph nodes*	X Spinal cord*
X Duodenum*	XX Spleen	
X Jejunum*	X Thymus*	Glands
X Ileum*	X Eyes (optic nerve)*	X Harderian glands
X Cecum*		X Thyroids*
X Colon*	Urogenital System	X Seminal vesicle
X Rectum*	XX Kidneys*	X Parathyroids*
XXLiver*	X Urinary bladder*	X Mammary gland*
X Gallbladder*	XX Testes*	XX Adrenals*
	X Ovaries*	X Pancreas*
	X Uterus*	XX Epididymides
	x Vagina*	X Lacrimal gland
		X Prostate
		X Pituitary*

Other	Respiratory System
X Bone (sternum, femur with joint)*	X Trachea*
X Head	X Lung*
X Skeletal muscle*	X Larynx and pharynx
X Skin*	
X All gross lesions and masses*	

(a) Organ weights and organ-to-body-weight ratios

Results: Treatment-related increases in absolute mean liver weights were observed in mid-high and high-dose males and females that survived until study termination (Table 3). These increases were statistically significant after liver weights were adjusted for body weights (115-136% of control in males and 109-117% of control in females). No histopathological changes, however, were observed in the liver in any of the test groups.

(b) Macroscopic Pathology

Results: Tables 4 and 5 summarize selected data on the incidence of macroscopic lesions. An increase in the incidence of forestomach excrescence was seen in animals treated with 600-PPM and 1200 ppm (8% and 10% in 600 ppm males and females, respectively, 24% and 30% in 1200 ppm males and females, respectively, and 0% and 2% in control males and females, respectively). A slight increase in the incidence of prominent/thickened limiting ridge of the forestomach was also observed in 1200 ppm males and 600 ppm and 1200 ppm females; however, the increase in the incidence was not dose related (16% in 1200 ppm males compared to 0% in controls and 16% and 10% in 600 ppm and 1200 ppm females, respectively, compared to 2% in controls). An increase in the incidence of minimal adipose tissue was observed in 1200 ppm males (30% compared to 12% in controls). Males treated with 600 ppm and 1200 ppm also and an increased incidence of lung masses (32% and 34%, respectively, compared to 18% in controls). An increased incidence of liver enlargement was observed in 1200 ppm females (12% compared to 0% in controls), and a slightly increased incidence of pale kidney was observed in 1200 ppm males (26% compared to 16% in controls).

(c) Microscopic pathology

Nonneoplastic Lesions: Tables 6 and 7 summarize selected data on nonneoplastic lesion incidences. A statistically significant increase in the incidence of minimal-to-marked papillomatous hyperplasia was seen in the nonglandular portion of the stomach (i.e., forestomach) in 1200 ppm males (20% incidence compared to 0% in controls for papillomatous hyperplasia and 6% in controls for epithelial hyperplasia). A nonsignificant increase of moderate-to-marked papillomatous hyperplasia was observed in high-dose females (8% compared to 0% in controls). A statistically significant increase in the incidence of minimal-to-moderate epithelial hyperplasia in the forestomachs was seen in 1200-ppm males (20% incidence compared to 6% in controls). These findings were considered to be treatment related.

A significant increase in the incidence of subscapular proliferations of fusiform cells of the adrenals was observed in females dosed at 30, 600 and 1200 ppm (86%, 90%, and 92%, respectively, compared to 68% in controls). However, the increase

in incidence was slight and not dose related and was therefore not considered to be a compound-related effect. Females dosed at 1200 ppm showed a significant increase of spleen hemosiderosis and hemorrhagic cysts of the ovaries (12% and 10%, respectively, compared to 0% in controls). Females dosed at 1200 ppm also showed a significant increase in incidence of parenchymal inflammatory cells of the liver (22% compared to 2% in controls). A significant increase in the incidence of amyloidosis occurred in the following organs and groups: adrenal (40% in 1200 ppm males compared to 20% in controls, duodenum (38% in 1200-ppm males compared to 18% in controls and 22% in 1200 ppm females compared to 32% in controls), jejunum (40% in 1200 ppm males compared to 22% in controls), ileum (30% in 1200 ppm males compared to 12% in controls). Amyloidosis is a common spontaneous finding, and no dose-related trends were evident; thus, it was considered to be of minor toxicological importance.

Neoplastic Lesions: Tables 8 and 9 summarize selected data on neoplastic lesions. Both males and females dosed at 1200 ppm showed significant increases in the incidence of squamous cell papillomas of the nonglandular portion of the stomach (12% in males compared to 0% in controls and 20% in females compared to 2% in controls). Males showed a significant trend in the incidence of squamous cell carcinomas of the nonglandular portion of the stomach. However, although males dosed at 1200 ppm showed a slight increase in incidence of this tumor (6% compared to 0% in controls), this was not significant upon pairwise comparison. A slight but not significant increase in the number of 600 ppm and 1200 ppm males that had pulmonary adenomas and/or adenocarcinomas was observed. Upon intergroup comparison of tumor incidence, a significant increase in the incidence of tumors was seen in males dosed at 600 ppm and 1200 ppm (46% and 48%, respectively, compared to 28% in controls). A slight increase (nonsignificant) in the incidence of tumors was also observed in females dosed at 1200 ppm (36% compared to 26% in controls). At 1200 ppm, there was a significant increase in the number of females with benign tumors (30% compared to 12% in controls). At 600 ppm, in males, there were significant increases in the incidence of malignant tumors and the number of animals with single tumors. At 1200 ppm, a slight, but not significant, increase in these parameters was observed in males.

B. DISCUSSION

This study is classified Core Guideline and satisfies the guideline requirements (83-2) for an carcinogenicity study in mice. Dosing was adequate and survival was acceptable in the study.

Under the conditions of the study, there was a statistically significant increase in the number of tumor-bearing males fed 600 ppm and 1200 ppm for 80 weeks. A slight increase (nonsignificant)

was observed in the number of tumor-bearing females dosed at 1200 ppm. Squamous cell tumors of the stomach were the most common treatment-related neoplastic lesion seen in the study. The first tumor, a squamous cell carcinoma of the stomach, was observed in a high-dose male that died after 62 weeks of treatment. Both males and females showed increases in the incidence of squamous cell papillomas of the nonglandular portion of the stomach. This finding was significant for animals at 1200 ppm. There was a significant trend in the incidence of squamous cell carcinomas of the nonglandular portion of the stomach in males. There was a significant trend in benign and/or malignant stomach tumors in both males and females. There was a significant increase in the number of females with benign tumors at 1200 ppm. A slight but not significant increase was observed in the number of males with pulmonary adenomas and/or adenocarcinomas at 600 ppm and 1200 ppm. However, this finding was not dose related, and the incidence was within the historical control range.

In addition to the increase in tumor incidence observed in the study, other effects indicative of stomach toxicity were observed. An increase in the incidence of forestomach excrescence was seen in males and females dosed at 600 ppm and 1200 ppm. There was a significant increase in the incidence of papillomatous hyperplasia and epithelial hyperplasia of the nonglandular portion of the stomach in high-dose males. In addition, changes indicative of hepatotoxicity were also observed. Treatment-related increases in absolute liver weight were observed in high-dose males and females that survived until study termination. There was an increased incidence of liver enlargement and parenchymal inflammatory cells of the liver in high-dose females.

The toxicity of CGA 154281 to the stomach observed in the current study is supported by signs of stomach toxicity observed in a combined chronic toxicity/carcinogenicity study in rats (MRID No. 428887-04). In the rat study, epithelial hyperplasia and hyperkeratosis of the forestomach were observed in females at the LOEL of 50 ppm (2.0 mg/kg/day in males and 2.8 mg/kg/day in females). In addition, at 500 ppm (20.6 mg/kg/day in males and 28.2 mg/kg/day in females) and above, an increased incidence of excrescences in the forestomach was observed in males. At the highest dose tested, 1000 ppm (41 mg/kg/day in males and 59 mg/kg/day in females), additional stomach lesions included raised areas on the epithelial aspect of the forestomach of both males and females; increased nodularity and papillomatous hyperplasia of the limiting ridge and epithelial hyperplasia and hyperkeratosis of the nonglandular stomach in males; and increased excrescences and papillomatous hyperplasia of the nonglandular stomach in females. Peto analysis revealed statistically significant trends for increases in the incidences of squamous cell papillomas in males and squamous cell carcinomas (and carcinomas and/or papillomas) in females at the doses tested under conditions of this study. However, the incidences of these tumors were not

significantly increased by pairwise comparison at any dose.

Effects other than stomach toxicity also occurred in the mouse study. Mean body weights were significantly decreased in high-dose males after week 7 of the study. Cumulative body weight gain was significantly decreased in high-dose males when compared to controls. Males dosed at 600 ppm and 1200 ppm also had an increased incidence of lung masses. There was also a slightly increased incidence of pale kidney in high-dose males. There were significantly increased incidences of spleen hemosiderosis and hemorrhagic cysts of the ovaries in the 1200 ppm females.

Based on increased incidences of forestomach excrescences, the LOEL for systemic toxicity was determined to be 600 ppm (75 and 93 mg/kg/day in males and females, respectively). The NOEL was 30 ppm (3.7 and 4.7 mg/kg/day in males and females, respectively). In addition, there were increases in the incidence of squamous cell papillomas of the nonglandular portion of the stomach at 1200 ppm in both males and females. There was also a significant trend in the incidence of squamous cell carcinomas of the nonglandular portion of the stomach in males. There was a significant trend in benign and/or malignant stomach tumors in both males and females.

The doses employed in this study were sufficient to produce a compound-related systemic effect and appeared to be adequate to test the carcinogenic potential of the test material.

TABLE 1. Mean Body Weight at Representative Intervals in Mice Fed CGA 154281 in the Diet for 80 Weeks[a]

Dietary Level (ppm)	Mean Body Weight (g ± S.D.) at Study Intervals (week)							
	0	12	24	36	48	60	72	80
				Males				
0	27±1.9[b]	38±3.5	42±4.9	45±5.5	47±6.0	48±5.8	48±5.8	47±5.8
10	28±1.9	39±3.0	43±4.6	47±5.2	48±5.7	48±6.0	47±5.5	47±5.5
30	28±1.9	38±4.0	44±5.0	48±6.1	49±7.1	50±7.1	50±7.4	49±7.7
600	27±2.3	38±3.3	42±4.9	45±6.3	46±6.1	47±6.2	46±5.8	45±6.1
1200	28±2.2	36±2.4**	40±3.0**	42±3.2**	43±4.2**	44±3.6**	44±4.2**	43±4.8**
				Females				
0	23±1.7	30±3.1	32±3.5	34±4.6	36±5.0	37±5.5	38±5.7	38±6.8
10	23±1.8	29±3.1	33±4.2	36±5.6	38±6.2	39±6.5	39±6.8	41±6.7
30	24±1.7	30±3.4	33±4.8	35±5.4	37±6.2	39±7.0	38±7.0	39±6.9
600	23±2.1	29±3.2	33±3.8	35±4.5	36±5.2	37±4.9	37±4.8	38±5.6
1200	22±1.7*	29±2.4	32±3.6	34±4.2	36±5.2	38±5.4	38±6.2	39±6.4

[a] Data extracted from Study No. CBG 510/911356, Table 4 (pp. 51-53) and Appendix 1.
[b] Standard deviations calculated by the reviewers.

* Significantly different from control, $p<0.05$.
** Significantly different from control, $p<0.01$.

TABLE 2. Mean Body Weight Gain at Representative Intervals in Mice Fed CGA 154281 for 80 Weeks[a]

Dietary Level (ppm)	Mean Body Weight Gain (g ± S.D.) at Study Intervals (week)			
	0 to 13	0 to 26	0 to 52	0 to 80
			Males	
0	11.3±2.80	15.7±4.12	20.5±5.47	19.9±5.28
10	11.6±2.70	15.8±4.11	20.3±5.33	19.0±5.20
30	11.3±3.58	16.9±4.93	22.8±6.68	21.8±7.71
600	10.5±3.09	15.1±4.79	19.6±5.84	17.8±5.56
1200	9.0±2.14**	12.7±2.79**	16.6±3.97**	15.5±4.30**
			Females	
0	7.0±2.71	10.0±3.45	13.1±4.58	15.5±6.57
10	6.7±3.07	9.9±4.40	14.5±6.25	17.7±6.45
30	6.4±3.48	9.5±4.53	12.9±6.52	15.4±7.19
600	7.1±2.98	10.5±3.83	13.2±4.45	14.8±5.13
1200	6.8±2.34	9.9±3.47	13.3±4.70	16.8±5.95

[a] Data extracted from Study No. GBG 510/911356, Table 5, p. 54.

** Significantly different from control, $p<0.01$.

TABLE 3. Mean Liver Weights (Nonadjusted and Adjusted for Final Body Weight) in Mice Fed CGA 154281 in the Diet for 80 Weeks[a,b]

Organ	Mean Liver Weights at Each Dietary Level (ppm)				
	0	10	30	600	1200
			Males		
Liver (g ± S.D.) Nonadjusted	2.46 ± 0.769	2.56 ± 0.732	2.63 ± 0.895	2.66 ± 0.587	3.17 ± 1.334
Liver (g) Adjusted	2.32	2.47	2.39	2.66* (115%)[b]	3.16** (136%)
			Females		
Liver (g ± S.D.) Nonadjusted	1.89 ± 0.288	1.99 ± 0.359	1.88 ± 0.488	2.04 ± 0.328	2.24 ±0.324
Liver (g) Adjusted	1.90	1.94	1.89	2.08* (109%)	2.23** (117%)

[a] Data extracted from Study No. CBG 510/911356, Table 11 (pp. 66 and 67).
[b] Numbers in parentheses represent percentage of average control value.

* Significantly different from control, $p<0.05$.
** Significantly different from control, $p<0.01$.

TABLE 4. Incidence of Selected Macroscopic Findings (%) in Male Mice Fed CGA 154281 for 80 Weeks[a,b,c]

Organ/ Lesion	Incidence at Each Dietary Level (ppm)				
	0	10	30	600	1200
Forestomach					
Excrescences	0	0	0	4 (8.0)	12 (24.0)
Limiting ridge--prominent/thickened	1 (2.0)	3 (6.0)	1 (2.0)	1 (2.0)	8 (16.0)
Adipose tissue					
Minimal	6 (12.0)	6 (12.0)	5 (10.0)	3 (6.0)	15 (30.0)
Lung					
Masses	9 (18.0)	13 (26.0)	11 (22.0)	16 (32.0)	17 (34.0)
Liver					
Enlarged	1 (2.0)	1 (2.0)	2 (4.0)	5 (10.0)	4 (8.0)
Kidney					
Pale	8 (16.0)	9 (18.0)	9 (18.0)	5 (10.0)	13 (26.0)

[a] Data extracted from Study No. CBG 510/911356, Table 12 (pp. 68-75) and Appendix 5.
[b] N=50 for each dose group; includes animals that died during study and animals that were sacrificed at study termination.
[c] Numbers in parentheses indicate percentage of animals in dose group with lesion.

TABLE 5. Incidence of Selected Macroscopic Findings (%) in Female Mice Fed CGA 154281 for 80 Weeks[a,b,c]

Organ/ Lesion	Incidence at Each Dietary Level (ppm)				
	0	10	30	600	1200
Forestomach					
Excrescences	1 (2.0)	0	0	5 (10.0)	15 (30.0)
Limiting ridge prominent/thickened	1 (2.0)	3 (6.0)	0	8 (16.0)	5 (10.0)
Adipose tissue					
Minimal	19 (38.0)	10 (20.0)	12 (24.0)	12 (24.0)	15 (30.0)
Liver					
Enlarged	0	2 (4.0)	3 (6.0)	2 (4.0)	6 (12.0)

[a] Data extracted from Study No. CBG 510/911356, Table 12 (pp. 68-75) and Appendix 5.
[b] N=50 for each dose group; includes animals that died during study and animals that were sacrificed at study termination.
[c] Numbers in parentheses indicate percentage of animals in dose group with lesion.

TABLE 6. Incidence of Selected Nonneoplastic Lesions (%) in Male Mice Fed CGA 154281 for 80 Weeks[a,b,c]

Organ/ Lesion	Incidence at Dietary Level (ppm)				
	0	10	30	600	1200
Stomach (nonglandular)					
Papillomatous hyperplasia					
Total	0	0	0	0	10 (20.0)**
Minimal	0	0	0	0	5*
Moderate	0	0	0	0	4
Marked	0	0	0	0	1
Epithelial hyperplasia					
Total	3 (6.0)	0	2 (4.0)	2 (4.0)	10 (20.0)*
Minimal	2	0	2	2	8*
Moderate	1	0	0	0	2
Duodenum					
Amyloidosis	9 (18.0)	14 (28.0)	8 (16.0)	9 (18.0)	19 (38.0)*
Jejunum					
Amyloidosis	11 (22.0)	9 (18.0)	8 (16.0)	8 (16.0)	20 (40.0)*

TABLE 6 (Continued). Incidence of Selected Nonneoplastic Lesions (%) in Male Mice Fed CGA 154281 for 80 Weeks[a,b,c]

Organ/ Lesion	Dietary Level (ppm)				
	0	10	30	600	1200
Ileum:					
Amyloidosis (moderate)	6 (12.0)	9 (18.0)	5 (10.0)	6 (12.0)	15 (30.0)*
Adrenal:					
Amyloidosis	10 (20.0)	12 (24.0)	7 (14.0)	8 (16.0)	20 (40.0)*

[a] Data extracted from Study No. CBG 510/911356, Table 14c (pp. 192-228) and Appendix 5.
[b] N=50 for each dose group; includes animals that died during study and animals that were sacrificed at study termination.
[c] Numbers in parentheses indicate percentage of animals in dose group with lesion.

* Significantly different from control, $p<0.05$.
** Significantly different from control, $p<0.01$.

TABLE 7. Incidence of Selected Nonneoplastic Lesions (%) in Female Mice Fed CGA 154281 for 80 Weeks[a,b,c]

Organ/ Lesion	Incidence at Each Dietary Level (ppm)				
	0	10	30	600	1200
Stomach (nonglandular)					
Papillomatous hyperplasia					
Total	0	0	0	1 (2.0)	4 (8.0)
Moderate	0	0	0	1	1
Marked	0	0	0	0	3
Epithelial hyperplasia					
Total	0	2 (4.0)	0	2 (4.0)	3 (6.0)
Minimal	0	1	0	2	3
Moderate	0	1	0	0	0
Kidney					
Glomerulonephritis	4 (8.0)	6 (12.0)	6 (12.0)	4 (8.0)	4 (8.0)
Duodenum					
Amyloidosis	16 (32.0)	11 (22.0)	9 (18.0)	14 (28.0)	11 (22.0)
Jejunum					
Amyloidosis	15 (30.0)	12 (24.0)	10 (20.0)	15 (30.0)	12 (24.0)
Ileum					
Amyloidosis (moderate)	12 (24.0)	13 (26.0)	8 (16.0)	10 (20.0)	10 (20.0)

TABLE 7 (Continued). Incidence of Selected Nonneoplastic Lesions (%) in Female Mice Fed CGA 154281 for 80 Weeks[a,b,c]

Organ/ Lesion	Incidence at Each Dietary Level (ppm)				
	0	10	30	600	1200
Adrenal					
Amyloidosis	16 (32.0)	14 (28.0)	10 (20.0)	13 (26.0)	15 (30.0)
Subscapular proliferation of fusiform cells	34 (68.0)	37 (74.0)	43 (86.0)*	45 (90.0)**	46 (92.0)**
Spleen					
Haemosiderosis	0	3 (6.0)	0	3 (6.0)	6 (12.0)*
Liver					
Parenchymal inflammatory cells	2 (4.0)	0	2 (4.0)	4 (8.0)	11 (22.0)**
Ovaries					
Haemorrhagic cyst(s)	0	2 (4.0)	2 (4.0)	2 (4.0)	5 (10.0)*

[a] Data extracted from Study No. CBG 510/911356, Table 14c (pp. 172-228) and Appendix 5.
[b] N=50 for each dose group; includes animals that died during study and animals that were sacrificed at study termination..
[c] Numbers in parentheses indicate percentage of animals in dose group with lesion.

* Significantly different from control, $p<0.05$.
** Significantly different from control, $p<0.01$.

TABLE 8. Incidence of Selected Neoplastic Lesions (%) in Mice Fed CGA 154281 for 80 Weeks[a,b,c]

Organ/ Lesion	Incidence of Each Dietary Level (ppm)				
	0	10	30	600	1200
			Males		
Stomach (nonglandular)					
Squamous cell papilloma	0	0	0	2 (4.0)	6 (12.0)*
Squamous cell carcinoma	0	1 (2.0)	0	1 (2.0)	3 (6.0)
Lung					
Pulmonary adenoma	8 (16.0)	7 (14.0)	3 (6.0)	9 (18.0)	9 (18.0)
Pulmonary adenocarcinoma	2 (4.0)	4 (8.0)	2 (4.0)	5 (10.0)	4 (8.0)
			Females		
Stomach (nonglandular)					
Squamous cell papilloma	1 (2.0)	0	0	1 (2.0)	10 (20.0)**
Squamous cell carcinoma	0	0	0	1 (2.0)	1 (2.0)
Lung					
Pulmonary adenoma	1 (2.0)	1 (2.0)	2 (4.0)	2 (4.0)	3 (6.0)
Pulmonary adenocarcinoma	2 (4.0)	0	2 (4.0)	1 (2.0)	1 (2.0)

[a] Data extracted from Study No. CBG 510/911356, Table 14a (pp. 78-87) and Appendix 5.
[b] N=50 for each dose group, except for 10 ppm female lungs where N=49.
[c] Numbers in parentheses indicate percentage of animals in dose group with lesion.

* Significantly different from control, $p<0.05$.
** Significantly different from control, $p<0.01$.

TABLE 9. Intergroup Comparison of Tumour Incidence (%) in Mice Fed CGA 154281 for 80 Weeks[a,b,c]

Parameter	Incidence at Each Dietary Level (ppm)				
	0	10	30	600	1200
			Males		
Number of Tumor Bearing Animals	14 (28.0)	21 (42.0)	17 (34.0)	23 (46.0)*	24 (48.0)*
Animals with Malignant Tumors	3 (6.0)	8 (16.0)	5 (10.0)	11 (22.0)*	9 (18.0)
Animals with Benign Tumors	13 (26.0)	17 (34.0)	14 (28.0)	15 (30.0)	18 (36.0)
Animals with Single Tumors	10 (20.0)	16 (32.0)	14 (28.0)	20 (40.0)*	17 (34.0)
			Females		
Number of Tumor Bearing Animals	13 (26.0)	10 (20.0)	14 (28.0)	11 (22.0)	18 (36.0)
Animals with Malignant Tumors	9 (18.0)	8 (16.0)	12 (24.0)	8 (16.0)	5 (10.0)
Animals with Benign Tumors	6 (12.0)	3 (6.0)	4 (8.0)	3 (6.0)	15 (30.0)*
Animals with Single Tumors	11 (22.0)	8 (16.0)	12 (24.0)	11 (22.0)	15 (30.0)

[a] Data extracted from Study No. CBG 510/911356, Table 14b (pp. 88-91) and Appendix 6.
[b] N=50 for each dose group.
[c] Numbers in parentheses indicate percentage of animals in dose group.

* Significantly different from control, $p<0.05$.

FINAL

DATA EVALUATION REPORT

CGA 154281

Study Type:
Chronic Oral Toxicity Study in Dogs

Study Title:
52 Week Oral (Capsule) Toxicity Study
in the Beagle

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
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Principal Reviewer Sara Lundgaard Date 5/4/94
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QA/QC Manager William L McLellan Date 5/4/94
William McLellan, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 3-09
Clement Number: 32
Project Officer: Caroline C. Gordon

Guideline Series 83-1: Chronic Oral Toxicity in Nonrodents

EPA Reviewer: ~~David Van Ormer~~ DAVID S Liem, Ph.D. Signature: David Sliem
~~Registration Section, TOX II, HED~~ Toxicology Branch 2 Date: 9/6/95
~~Chemical Coordination Branch~~
Health Effects Division

EPA Section Head: ~~Albin Kocialski, Ph.D.~~ K. Clark Swentzel Signature: K Clark Swentzel
~~Registration Section~~, Toxicology Branch 2 Date: 9/6/95
~~Chemical Coordination Branch~~
Health Effects Division

DATA EVALUATION REPORT

STUDY TYPE: Chronic oral toxicity study in dogs

TEST MATERIAL: CGA 154281

SYNONYM: Benoxacor

P.C. NO.: 126101

TOX. CHEM. NO.: N/A

MRID NO.: 428887-01

STUDY NO.: 6904-380/158

SPONSOR: Ciba-Geigy Limited
Plant Protection Division
Safety Evaluation, PP2.56
CH-4002 Basle, Switzerland

TESTING FACILITY: Hazleton UK
Harrogate, England HG3 1PY

TITLE OF REPORT: 52 Week Oral (Capsule) Toxicity Study in the Beagle

AUTHOR: J.D. Wood

REPORT ISSUED: September 7, 1992

QUALITY ASSURANCE: A signed Quality Assurance Statement (September 7, 1992) and a signed GLP Certification Statement (dated September 7, 1992) were provided. The study was conducted in compliance with OECD guideline number 452 and EPA GLP regulations.

CONCLUSIONS: CGA 154281 was administered orally in gelatin capsules to male and female beagle dogs (4/sex/group) at doses of 0, 1, 5, 40, and 80 mg/kg/day for 52 weeks.

NOEL (systemic toxicity) = 5 mg/kg/day

LOEL (systemic toxicity) = 40 mg/kg/day based on decreases in mean body weight gain in males, increases (both sexes) in adjusted liver and kidney weights, and increased lipofuscin deposition in the kidney (both sexes).

At 80 mg/kg/day, a slight increase in incidence and severity of pigmentation in the proximal tubule of the kidney in males was observed. In addition, at top dose there was an indication of hemolytic anemia, expressed by decreased red cell parameters, and increased bilirubin (and increased spleen weight in two males).

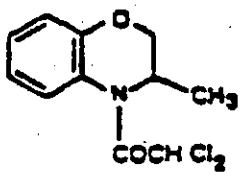
CORE CLASSIFICATION: Core Guideline. This study satisfies the guideline requirements (83-1) for a chronic oral toxicity study in dogs.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: CGA 154281

Structure:



Composition: 4-Dichloroacetyl-3,4-dihydro-3-methyl-2H-1,4-benzoxazine

Batch number: SG 7505-11

Purity: 96.8%

Physical property: Brown/grey powder

Stability: Not reported

Storage: Ambient temperature in the dark

2. Test Substance Analyses for Purity and Stability

The appropriate amount of the test article was weighed out weekly for each animal, placed in type I blank gelatin capsules (apparently a vehicle was not used), and administered orally 7 days a week for 52 weeks. Individual doses were adjusted weekly based on most recent body weight. Control animals received empty capsules as a treatment. A 10-g sample was returned to the sponsor for confirmation of test article identity before initiation of the study. This analysis was repeated with the remaining test article (90 g) at the termination of the study. The sponsor confirmed the identity and reported purities of 97.0% and 98.0%, respectively, for the two analyses.

3. Animals

Beagle dogs, 20 males and 20 females, were received from Hazleton Research Products, Inc. Animals were vaccinated before shipment and re-vaccinated upon arrival. Animals were housed in individual cages

during the working day and, when possible, in pairs of the same sex during the night. Animals were kept in environmentally controlled rooms and were acclimated to laboratory conditions for a minimum of 3 weeks. The rooms were maintained at a temperature of 16-22°C with a relative humidity of 40-80% and a 12-hour light/dark cycle. Before study initiation, their health status was reassessed and their suitability for test purposes was confirmed. The animals were then assigned randomly to groups (4/sex/group) such that no significant difference in average group weights existed. Animals were uniquely identified by tattoos. The dogs were 6-7 months of age at the initiation of dosing, and body weight ranges were 6.35-9.80 kg (males) and 5.45-7.75 kg (females). SQC Laboratory Diet A (Expanded) pellets (400 g/day) and filtered tap water (*ad libitum*) were provided throughout the study. Food and water were analyzed for environmental contaminants. Caging and sanitary conditions were maintained in accordance with the SOPs of the testing facility.

The dogs (4/sex/group) were assigned to the following test and control groups:

Group	Dose Levels (mg/kg/day)	Number of Animals Males	Females
1 Control	0	4	4
2 Low	1	4	4
3 Low-intermediate	5	4	4
4 High-intermediate	40	4	4
5 High	80	4	4

4. Rationale for Dose Selection

Doses were based on the results from a 28-day range-finding study in dogs (HUK project number 380/171, Ciba-Geigy project number 89 1410). The results of this study were provided in study number 6904-380/158. Four male and 4 female beagle dogs (1/sex/group) were administered daily doses of 0, 100, 200, or 300 mg/kg/day by capsule. Treatment at the two highest doses was stopped after 7 days because of reduction in food consumption, vomiting, and reduced body weight gain. Animals that received 100 mg/kg/day showed a reduction in food consumption. Because dose levels of 100 mg/kg/day or more caused adverse effects, the control animals were given 80 mg/kg/day for 7 days during which time no effects were seen. The high dose in the chronic study was based on the maximum tolerated dose in the pilot study (80 mg/kg/day).

5. Statistical Analyses

All data, except for organ weight data, were analyzed by two-way analysis of variance (ANOVA). When significant differences were seen, Dunnett's test was performed to determine group differences. Significant differences in pre-dose variables were analyzed by using a protected t-test if the p value of the ANOVA was less than 0.05. Post-dose variables were analyzed for dose-response relationships by using a linear regression. Levene's test for equality of variances across groups, between sexes, and for interactions was also performed. When this test showed evidence of group effects or a sex-group interaction, the data were re-analyzed using Kruskal-Wallis (ANOVA), Wilcoxon rank sum test (pairwise comparisons), and the Terpstra-Jonckheere test (dose response). Organ weights were analyzed by using analysis of covariance (ANCOVA) to adjust for terminal body weight.

6. General Observations

(a) Mortality/moribundity/survival

Animals were observed daily for mortality and moribundity.

Results: All animals survived until study termination.

(b) Clinical signs

Observations for adverse clinical effects were made once daily throughout the study.

Results: Clinical observations are summarized in the table below. The only clinical sign that appeared to be affected by the intake of CGA 154281 was thinness. This symptom was observed in 1/4 control females, 1/4 males dosed with 1 mg/kg/day, 2/4 males dosed with 5 mg/kg/day, 3/4 males dosed with 40 mg/kg/day, 3/4 males dosed with 80 mg/kg/day, and 2/4 females dosed with 80 mg/kg/day. Although there was a tendency towards thinness in the males dosed at 40 and 80 mg/kg/day, the occurrence of this sign was sporadic and was not seen consistently throughout the study in individual animals (for example, occurred in 1 high-dose male during weeks 4-8 and in another during weeks 12-39). Weight loss and persistent diarrhea were observed in 1 male dosed with 5 mg/kg/day. The animal was treated and maintained on study, but the data for this animal were excluded from group means and statistical analyses.

	Incidence of Thinness for Each Dose Level (mg/kg/day)				
Sex	0	1	5	40	80
Males	0/4	1/4	2/4	3/4	3/4
Females	1/4	0/4	0/4	0/4	2/4

[a]Data extracted from Study No. 6904-380/158, Appendix 1.1 (pp. 85-89).

(c) Body weights/body weight gains/food consumption

Body weights/body weight gains--Body weights were recorded once weekly throughout the study.

Results: Mean body weight and mean body weight gain data are summarized in Tables 1 and 2. No statistically significant changes in mean body weights were observed. However, absolute body weights in males dosed with 40 and 80 mg/kg/day were consistently decreased when compared to controls throughout the study (88-98% of controls). At the termination of the study, mean body weights of males dosed with 40 mg/kg/day and males dosed with 80 mg/kg/day were 90% of the mean body weight for controls. Statistically significant decreases in mean body weight gain were observed in males dosed at 80 mg/kg/day during weeks 13-26 (9% of control value). No other statistically significant changes were observed in body weight gain; however, cumulative body weight gain for study weeks -1-52 was decreased (not significant) in males dosed at 40 and 80 mg/kg/day when compared to controls (64% and 61% of control value, respectively). Although body weight gain was consistently decreased throughout the study (except during weeks 26-39) in males dosed at 80 mg/kg/day, body weight gain decreases were more dramatic in males dosed at 40 mg/kg/day after study week 26. Female weight gains at the top two doses (40 and 80 mg/kg/day) were 76% and 85% of control value.

TABLE 1. Mean Body Weight at Representative Intervals in Dogs Orally Administered CGA 154281 for 52 Weeks[a]

Dose (mg/kg/day)	Mean Body Weight (kg ± S.D.) at Study Intervals					
	1 week	10 weeks	20 weeks	30 weeks	40 weeks	52 weeks
			Males			
0	8.74±0.96	10.18±1.16	10.51±1.10	11.01±1.20	11.31±1.34	11.18±1.46
1	8.41±1.21	10.08±1.11	10.41±1.36	10.86±1.35	11.06±1.41	11.01±1.20
5	8.17±1.46	9.63±1.59	10.10±1.68	10.43±1.77	10.67±1.60	10.55±2.17
40	8.60±0.64	9.78±1.10	10.29±1.34	10.44±1.17	10.40±1.17	10.11±0.97
80	8.73±0.90	9.51±1.12	9.79±1.25	10.13±1.41	10.18±1.29	10.11±1.31
			Females			
0	6.75±0.79	7.88±0.81	8.21±0.92	8.56±0.89	8.65±1.06	8.59±1.33
1	7.11±0.90	8.44±1.11	9.33±0.80	9.31±0.78	9.59±0.75	9.35±0.64
5	7.03±1.00	8.13±1.18	8.58±1.08	8.70±1.28	8.86±1.10	8.85±1.12
40	6.98±0.57	7.85±0.83	8.36±0.91	8.36±0.84	8.44±1.09	8.45±1.04
80	6.73±0.90	7.63±1.04	8.03±1.35	8.19±1.22	8.55±1.57	8.39±1.35

[a]Data extracted from Study No. 6904-380/158, Table 1.1 (pp. 42-45).

TABLE 2. Mean Body Weight Gain at Representative Intervals in Dogs Orally Administered CGA 154281 for 52 Weeks[a]

Dose (mg/kg/day)	Mean Body Weight Gain (kg ± S.D.) at Study Intervals				
	-1-13 weeks	13-26 weeks	26-39 weeks	39-52 weeks	-1-52 weeks
			Males		
0	1.79±0.38	0.69±0.24	0.28±0.34	-0.04±0.13	2.71±0.87
1	2.21±0.67	0.55±0.55	0.16±0.03	0.04±0.33	2.96±0.48
5	1.62±0.12	0.80±0.41	0.23±0.23	-0.05±0.54	2.60±0.82
40	1.61±0.99	0.36±0.29	-0.01±0.31	-0.23±0.36	1.74±0.73
80	1.26±0.63	0.06±0.55*	0.38±0.17	-0.06±0.11	1.64±0.98
			Females		
0	1.60±0.46	0.36±0.48	0.20±0.20	-0.05±0.50	2.11±1.34
1	1.80±0.36	0.59±0.47	0.36±0.44	-0.29±0.31	2.46±0.32
5	1.13±0.24	1.01±0.39	-0.10±0.26	0.03±0.37	2.06±0.61
40	1.10±0.49	0.39±0.26	0.16±0.18	-0.05±0.20	1.60±0.48
80	1.30±0.29	0.09±0.28	0.55±0.46	-0.15±0.37	1.79±0.55

[a]Data extracted from Study No. 6904-380/158, Tables 1.1 and 1.2 (pp. 45-47).

*Significant using the dose-response test ($p<0.05$)

Food consumption--Food consumption was measured daily for each dog and weekly averages were calculated (g/animal/week).

Results: No treatment-related effects on food consumption were observed.

(d) Ophthalmoscopic examination

Ophthalmic examinations were conducted on all animals prior to the initiation of dosing and during weeks 12, 25, and 52 of the study. Examinations were conducted using a Keeler ophthalmoscope. Before examination, 1.0% tropicamide was placed in each eye.

Results: No treatment-related ocular lesions were observed.

(e) Auditory response

The auditory response of each animal was examined prior to the initiation of dosing and during weeks 13, 26, and 52.

Results: No treatment-related effects were observed in the auditory response of animals.

(f) Physical examination

Tests examining behavior, body position/posture, gait, muscle tone, respiration, skin, appendages, nails and foot pads, urogenital and anal areas, mucous membranes, eyes, ears, nose, gums, and teeth were conducted on all animals during study weeks 13, 26, and 52.

Results: No treatment-related effects on the parameters examined were observed.

7. Clinical Pathology

Blood samples were obtained from all animals prior to initiation of the study and during study weeks 13, 26, and 52. Because of technical problems, additional blood samples were collected during weeks 14 and 15. Blood samples were collected from the jugular veins of animals. Animals were fasted overnight prior to collection of blood samples. The hematology and clinical chemistry parameters indicated by an "X" below were examined:

(a) Hematology

Hematocrit (HCT)	X Leukocyte differential count
X Hemoglobin (HGB)*	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)*	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC)*	X Mean corpuscular volume (MCV)
X Platelet count*	X Prothrombin time
X Heinz-body determinations	X Activated partial thromblastin time (APTT)
X Reticulocyte count (RETIC)	
X Packed cell volume (PCV)	

*Recommended by Subdivision F (November 1984) Guidelines

Results: Selected hematology data are summarized in Tables 3 and 4. The following statistically significant changes were observed in animals at various intervals throughout the study: decreases in red blood cell count in males dosed at 80 mg/kg/day (89-91% of control value); decreases in hemoglobin in males dosed at 40 and 80 mg/kg/day (86-90% of control value) and in females dosed at 80 mg/kg/day (92% of control value); decreases in packed cell volume in males dosed at 40 and 80 mg/kg/day (86-90% of control value); decreases in mean cell volume and mean corpuscular hemoglobin in males dosed at 80 mg/kg/day (96% and 95% of control value, respectively); and increases in platelet count in females dosed at 40 and 80 mg/kg/day (135-150% of control value).

(b) Blood (clinical) chemistry

Electrolytes	Other
X Calcium*	X Albumin*
X Chloride*	X Albumin/globulin ratio
Magnesium	X Blood creatinine*
X Phosphorus*	X Blood urea nitrogen*
X Potassium*	X Cholesterol*
X Sodium*	X Globulins
	X Glucose*
Enzymes	X Total bilirubin*
	Direct bilirubin
X Alkaline phosphatase	X Total protein*
X Serum alanine aminotransferase (SGPT)*	X Triglycerides
X Serum aspartate aminotransferase (SGOT)*	
X Gamma glutamyl transpeptidase	
X Creatinine phosphokinase*	

*Recommended by Subdivision F (November 1984) Guidelines

Results: Selected clinical chemistry data are summarized in Tables 5 and 6. Bilirubin levels were significantly decreased at pre-dose when compared to controls (71-79% of control value) in the animals that were designated to receive 1, 40, and 80 mg/kg/day. By week 13, bilirubin levels were significantly

TABLE 3. Selected Hematology Parameters in Male Dogs Orally Administered CGA 154281 for 52 Weeks[a]

Parameter	Hematology Values at Each Dose Level (mg/kg/day)				
	0	1	5	40	80
RBC (E6/CMM)					
Pre-dose	6.13±0.12	5.73±0.22	5.96±0.41	6.22±0.68	6.02±0.75
Week 13	7.05±0.46	7.12±0.46	6.34±0.62	6.68±0.65	6.41±0.40*
Week 26	7.10±0.41	7.32±0.10	6.63±0.16	6.77±0.61	6.35±0.62*
Week 52	7.25±0.37	7.43±0.28	6.79±0.58	6.58±0.56	6.52±0.61**
HGB (G/DL)					
Pre-dose	14.4±0.4	13.0±0.8	14.0±0.6	14.3±1.5	13.6±1.6
Week 13	16.5±1.1	16.3±1.1	15.0±0.8	15.4±1.2	14.9±1.1*
Week 26	16.8±1.1	17.2±0.7	16.0±0.6	15.8±1.3	14.6±1.4**
Week 52	17.6±0.9	17.7±0.9	16.7±0.8	15.6±1.5*	15.1±1.6**
PCV (%)					
Pre-dose	42.8±1.0	38.9±2.2	41.3±1.8	42.9±4.2	40.5±4.5
Week 13	49.0±3.0	48.7±3.0	44.4±2.4	45.4±3.8	44.0±3.0*
Week 26	49.2±3.6	49.9±1.6	46.9±1.6	45.7±3.9	42.8±3.9*
Week 52	49.9±3.0	50.3±2.2	47.2±2.3	44.4±4.0*	43.1±4.1**
PLT (E3/CMM)					
Pre-dose	354±65	365±81	364±121	407±68	314±60
Week 13	306±77	329±72	358±41	394±49	343±85
Week 26	323±44	333±71	355±86	404±35	359±77
Week 52	311±21	337±94	345±96	405±15	387±103

TABLE 3 (Continued). Selected Hematology Parameters in Male Dogs Orally Administered CGA 154281 for 52 Weeks[a]

Parameter	Hematology Values at Each Dose Level (mg/kg/day)				
	0	1	5	40	80
MCV (FL)					
Pre-dose	69.8±1.6	67.8±1.6	69.4±3.1	69.1±1.0	67.4±1.4
Week 13	69.5±1.6	68.4±1.2	70.2±3.1	68.1±1.9	68.6±1.0
Week 26	69.2±1.5	68.2±1.3	70.7±2.9	67.5±2.1	67.3±1.0
Week 52	68.9±1.1	67.7±1.4	69.8±3.3	67.5±2.1	66.2±0.5*
MCH (PG)					
Pre-dose	23.5±0.4	22.6±0.6	23.6±1.0	22.9±0.6	22.6±0.4
Week 13	23.5±0.4	22.9±0.3	23.7±1.0	23.1±0.8	23.2±0.4
Week 26	23.6±0.2	23.5±0.7	24.1±1.0	23.4±0.9	23.0±0.5
Week 52	24.3±0.4	23.9±0.5	24.6±1.2	23.7±0.8	23.2±0.4*
MCHC (G/DL)					
Pre-dose	33.6±0.4	33.3±0.1	33.9±0.1	33.2±0.6	33.5±0.4
Week 13	33.7±0.4	33.4±0.3	33.7±0.1	33.9±0.4	33.8±0.3
Week 26	34.1±0.4	34.4±0.5	34.1±0.5	34.6±0.2	34.2±0.4
Week 52	35.3±0.4	35.3±0.4	35.3±0.1	35.1±0.2	35.1±0.5

[a]Data extracted from Study No. 6904-380/158, Table 3 (pp. 54-63).

*Significantly different using the dose-response test ($p \leq 0.05$)
**Significantly different using the dose-response test ($p \leq 0.01$)

TABLE 4. Selected Hematology Parameters in Female Dogs Orally Administered CGA 154281 for 52 Weeks*

Parameter	Hematology Values at Each Dose Level (mg/kg/day)				
	0	1	5	40	80
RBC (E6/CMM)					
Pre-dose	6.35±0.56	6.16±0.14	5.83±0.44	6.05±0.28	5.80±0.66
Week 13	6.93±0.60	6.55±0.53	6.93±0.29	6.59±0.13	6.43±0.36
Week 26	7.14±0.35	6.16±0.27	7.01±1.01	6.45±0.17	6.51±0.38
Week 52	7.21±0.42	6.90±0.32	7.14±0.52	6.92±0.39	6.64±0.28
HGB (G/DL)					
Pre-dose	14.8±1.6	14.0±0.5	13.6±0.8	13.9±0.9	13.4±1.5
Week 13	16.3±1.7	15.1±0.9	16.2±0.5	15.4±0.1	15.1±1.0
Week 26	17.1±0.6	14.7±0.7	16.6±2.1	15.3±0.4	15.4±0.8
Week 52	17.5±1.2	16.4±0.7	17.4±1.0	16.4±0.8	15.8±0.5*
PCV (%)					
Pre-dose	43.8±4.9	41.7±1.4	40.4±2.4	41.5±2.6	39.7±3.9
Week 13	48.2±4.9	44.8±2.7	47.8±1.1	45.7±0.7	44.7±2.9
Week 26	49.6±1.7	42.3±1.6	48.7±6.1	44.8±0.9	45.0±2.6
Week 52	49.3±3.2	46.6±1.4	49.3±2.7	46.9±1.9	45.1±1.2
PLT (E3/CMM)					
Pre-dose	301±30	361±89	309±22	297±95	391±83
Week 13	300±41	305±36	301±22	337±9	449±115*
Week 26	304±39	295±50	318±43	350±33	439±87*
Week 52	323±72	327±49	324±34	437±29*	438±70

TABLE 4 (Continued). Selected Hematology Parameters in Female Dogs Orally Administered CGA 154281 for 52 Weeks[a]

Parameter	Hematology Values at Each Dose Level (mg/kg/day)				
	0	1	5	40	80
MCV (FL)					
Pre-dose	68.8±1.9	67.6±1.6	69.4±1.4	68.6±1.3	68.6±1.7
Week 13	69.5±1.1	68.5±2.1	69.0±1.5	69.4±1.7	69.5±2.0
Week 26	69.5±2.0	68.7±1.7	69.7±1.6	69.5±1.7	69.1±1.9
Week 52	68.4±1.7	67.6±1.6	69.0±1.3	67.9±1.8	68.0±1.8
MCH (PG)					
Pre-dose	23.2±0.6	22.7±0.7	23.4±0.6	23.0±0.5	23.1±0.6
Week 13	23.4±0.4	23.1±0.8	23.3±0.6	23.4±0.4	23.5±0.7
Week 26	24.0±0.9	23.9±0.7	23.8±0.4	23.8±0.5	23.7±0.5
Week 52	24.3±0.7	23.8±0.5	24.4±0.4	23.7±0.5	23.8±0.5
MCHC (G/DL)					
Pre-dose	33.7±0.1	33.5±0.2	33.6±0.3	33.5±0.2	33.6±0.5
Week 13	33.8±0.2	33.7±0.2	33.8±0.3	33.7±0.4	33.9±0.1
Week 26	34.5±0.3	34.7±0.5	34.1±0.3	34.2±0.3	34.3±0.2
Week 52	35.5±0.2	35.1±0.5	35.3±0.2	34.9±0.5	35.0±0.2*

[a]Data extracted from Study No. 6904-380/158, Table 3 (pp. 54-63).

*Significantly different using the dose-response test ($p \leq 0.05$)
**Significantly different using the dose-response test ($p \leq 0.01$)

TABLE 5. Selected Clinical Chemistry Parameters from Male Dogs Orally Administered CGA 154281 for 52 Weeks[a]

Parameter	Clinical Chemistry Values at Each Dose Level (mg/kg/day)				
	0	1	5	40	80
CPK (IU/L)					
Pre-dose	281±63	231±69	259±124	251±85	238±100
Week 13	199±97	136±33	134±46	135±22	108±9*
Week 26	227±34	150±21**	146±12**	156±11**	140±23***
Week 52	137±22	136±26	119±37	173±45	147±58
GGT (IU/L)					
Pre-dose	4±1	5±2	7±2	6±2	6±1
Week 13	4±2	7±2	7±4*	6±3	6±1
Week 26	2±2	3±2	4±1	3±2	1±2
Week 52	4±1	3±1	5±1	5±2	5±1
CREATININE (μMOL/L)					
Pre-dose	71±12	69±4	70±9	62±8	63±10
Week 13	81±9	78±10	79±7	71±8	71±5*
Week 26	96±9	88±6	84±5	75±7**	80±8*
Week 52	86±7	83±12	79±9	69±4	77±6*
BILIRUBIN(μMOL/L)					
Pre-dose	2.4±0.4	1.8±0.3**	2.1±0.4	1.7±0.2**	1.9±0.2*
Week 13	2.0±0.6	1.8±0.3	1.9±0.2	1.9±0.2	2.6±0.4*
Week 26	2.9±0.3	2.6±0.6	2.6±0.5	2.8±0.7	3.4±0.9
Week 52	2.4±0.2	2.5±0.3	2.7±0.4	2.7±0.5	3.2±0.6*

[a]Data extracted from Study No. 6904-380/158, Table 4 (pp. 64-72).

*Significantly different using the dose-response test ($p \leq 0.05$)
**Significantly different using the dose-response test ($p \leq 0.01$)

TABLE 6. Selected Clinical Chemistry Parameters from Female Dogs Orally Administered CGA 154281 for 52 Weeks[a]

Parameter	Clinical Chemistry Values at Each Dose Level (mg/kg/day)				
	0	1	5	40	80
GPK (IU/L)					
Pre-dose	252±49	220±59	292±39	224±33	254±62
Week 13	153±54	127±18	194±30	132±39	169±34
Week 26	182±45	149±23	165±33	152±68	146±37
Week 52	199±81	163±58	157±40	123±37	126±19*
GGT (IU/L)					
Pre-dose	6±2	6±2	6±1	6±1	7±1
Week 13	5±1	6±1	6±2	5±1	7±1
Week 26	2±2	1±2	3±2	3±2	2±2
Week 52	4±2	3±2	5±1	5±2	5±1
CREATININE (µMOL/L)					
Pre-dose	68±5	65±7	67±5	65±3	67±8
Week 13	77±9	76±6	76±8	72±3	72±9
Week 26	81±14	87±7	82±11	75±7	80±6
Week 52	84±15	100±15	79±5	71±8	70±9*
BILIRUBIN(µMOL/L)					
Pre-dose	2.3±0.4	1.8±0.3*	2.1±0.2	1.8±0.2*	1.8±0.2*
Week 13	2.4±0.5	1.8±0.3	2.1±0.2	2.3±0.4	2.9±0.4*
Week 26	2.8±0.6	2.4±0.2	2.6±0.3	3.2±0.6	3.7±0.1*
Week 52	2.8±0.3	2.6±0.4	2.7±0.6	3.6±0.5	3.5±0.4**

[a]Data extracted from Study No. 6904-380/158, Table 4 (pp. 64-72).

*Significantly different using the dose-response test ($p \leq 0.05$)
**Significantly different using the dose-response test ($p \leq 0.01$)

increased in the groups that received 80 mg/kg/day (121-133% of control value). Creatinine levels were significantly decreased in males that received 40 and 80 mg/kg/day and in females that received 80 mg/kg/day (78-90% of control values). Creatinine phosphokinase activity was significantly decreased in males that received 1, 5, 40, and 80 mg/kg/day and in females that received 80 mg/kg/day (54-69% of control value). In both sexes, individual values of these parameters were highly variable. Thus, the decreases in creatinine levels and creatinine phosphokinase activity are not considered to be biologically significant. Significant changes occurred in other parameters, but these changes were sporadic and the values were within historical control ranges and, thus, the changes were not considered to be biologically significant.

(d) Urinalysis

Urinalyses were conducted during weeks 13, 26, and 52. Samples were collected through direct catheterization of the bladder. The parameters checked (X) below were examined.

X Appearance*	X Sediment (microscopic)	X Bilirubin*
Volume*	X Protein*	X Blood*
X Specific gravity*	X Glucose*	Nitrate
X pH*	X Ketones*	X Urobilinogen
X Reducing substances		

*Recommended by Subdivision F (November 1984) Guidelines

Results: No treatment-related changes in urinalysis parameters were observed.

8. Sacrifice and Pathology

Complete gross examinations were performed on all animals at terminal sacrifice. Animals were fasted overnight and then sacrificed by exsanguination following an overdose of sodium thiopentone. Tissues were preserved in 10% neutral buffered formalin (except for the eyes which were preserved in Davidson's fluid). Tissues marked with an "X" below were examined histologically in all animals. Organs indicated by "XX" below were also weighed for all animals.

Digestive System	Cardiovascular/Hematologic	Neurologic
X Tongue	X Aorta*	XX Brain
X Salivary glands*	XX Heart*	X Peripheral nerve (sciatic nerve)*
X Esophagus*	X Bone marrow*	X Spinal cord (three levels)
X Stomach*	X Lymph nodes*	XX Pituitary*
X Duodenum*	XX Spleen	X Eyes (optic nerve)*
X Jejunum*	X Thymus	
X Ileum*		Glandular
X Cecum*	Urogenital	
X Colon*		XX Adrenals*
X Rectum	XX Kidneys*	Lacrimal gland
XX Liver*	X Urinary bladder*	X Mammary gland
X Gallbladder*	XX Testes*	XX Thyroids*
X Pancreas*	XX Epididymides	X Parathyroids*
	X Prostate	Harderian glands
Respiratory	Seminal vesicle	
	XX Ovaries	
X Trachea*	X Uterus	
X Lung*	X Vagina	

Other

X Bone (sternum and rib)*
X Skeletal muscle (thigh)*
X Skin
X All gross lesions and masses

*Recommended by Subdivision F (November 1984) Guidelines

(a) Organ weights and adjusted organ weights

Results: Selected organ weight data are summarized in Table 7. Organ weights were analyzed against necropsy body weights by ANCOVA to produce "adjusted" weights. All adjusted liver weights (each sex) were above control values. At the 40 and 80 mg/kg/day dosages, respectively, adjusted liver weights were 41.9% and 32.0% (males), and 41.8% and 30.2% (females) above adjusted control values. A treatment effect is apparent, falling off somewhat at the top dose.

Adjusted kidney weights for each sex (particularly in males) were also above control values. At the 40 and 80 mg/kg/day dosages, the values for males were 21.8% and 20.4% (respectively) above controls.

(b) Macroscopic pathology

Results: No treatment-related macroscopic lesions were apparent.

(c) Microscopic pathology

Results: Pigment deposition in the kidneys of all animals was described as minimal except for 3/4 in the high-dose males that were described as slight (slight is considered more severe than

TABLE 7. Selected Organ Weight Data from Dogs Orally Administered CGA 154281 for 52 Weeks[a]

Parameter	Adjusted[b] and Absolute[c] Organ Weight Data for Each Dose Level (mg/kg/day)				
	0	1	5	40	80
			Males		
Liver (g)	296.9 (314.1±14.0)	327.5 (334.6±50.1)	304.0 (298.6±71.1)	421.3** (414.6±53.3)	392.0* (378.6±54.2)
Kidney (g)	50.408 (52.866±6.997)	52.299 (53.310±6.854)	56.188 (55.416±8.711)	61.400* (60.438±7.829)	60.749 (58.822±4.300)
			Females		
Liver (g)	249.8 (246.3±32.4)	250.3 (270.7±60.6)	280.8 (286.2±51.2)	354.2* (345.1±67.5)	325.3 (312.1±48.5)
Kidney (g)	37.457 (37.089±3.045)	43.124 (45.247±6.905)	38.352 (38.923±3.617)	44.443 (43.497±3.336)	43.442 (42.063±7.003)

[a]Data extracted from Study No. 6904-380/158, Table 5 (pp. 73-74).
[b]Group mean organ weights adjusted to overall mean necropsy body weight (g)
[c]Absolute group mean organ weight (g ± S.D.); calculated by reviewers.

*Significantly different from control value ($p \leq 0.05$)
**Significantly different from control value ($p \leq 0.01$)

minimal). The incidences of pigment deposition in the kidney are as follows:

Incidence of Pigment Deposition for Each Dose Level (mg/kg/day)					
Sex	0	1	5	40	80
Males	1/4	2/4	1/4	2/4	4/4
Females	1/4	1/4	0/4	2/4	2/4

[a]Data extracted from Study No. 6904-380/158, Table 6.2 (p.81) and Appendix 11 (pp. 211-261).

A fibrosarcoma was observed in the diaphragm of 1 female that received 5 mg/kg/day. This finding was considered incidental as the neoplasm is uncommon and no other neoplasms were observed in animals that received higher doses.

B. DISCUSSION

The data presented in this study show that administration of CGA 154281 had slight adverse effects on the kidney and on body weights in beagle dogs. The compound produced adverse renal effects in both sexes dosed at 40 and 80 mg/kg/day. An increase in the incidence and severity of deposition of pigment in the proximal tubules in the kidneys was observed in these animals, particularly in the high-dose males. In addition, absolute body weights in males dosed with 40 or 80 mg/kg/day were consistently decreased, although not significantly, when compared to controls throughout the study. Cumulative body weight gain (weeks -1-52) was decreased (not significant) in males dosed with 40 or 80 mg/kg/day when compared to controls. Because no subsequent changes in food consumption were observed, the decreases in body weight gain were considered to be treatment related.

In addition, increases in absolute and adjusted liver and kidney weights were observed in males and females that received 40 and 80 mg/kg/day. Adjusted liver weights at the high, mid, and top doses were increased over control values by 41.9% and 32.0% (males) and 41.8% and 30.2% (females). Adjusted male kidney weights at these two doses were elevated over control values by 21.8% and 20.4%, respectively. No microscopic lesions correlating with hepatotoxicity were observed. Changes in creatinine phosphokinase activity, gamma glutamyl transpeptidase levels, bilirubin levels, and creatinine levels also occurred, but these were not dose related and not consistent throughout the study and/or they were within historical control limits.

Changes in hematology parameters were also observed in the study. Statistically significant changes were observed (mainly in top dose males) and included decreases in red blood cell count, decreases in hemoglobin concentration, decreases in packed cell volume, decreases in mean cell volume, decreases in mean corpuscular hemoglobin concentration, and increases in platelet count. The depressions of red cell parameters, together with the small increased bilirubin (and the elevated spleen

weights in two high-dose males) may represent a tendency toward a compound-associated hemolytic disease.

The LOEL (based on decreases in body weight gain in males, increased liver and kidney weights, and kidney lipofuscin deposition) was determined to be 40 mg/kg/day. The NOEL was 5 mg/kg/day.

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FINAL

DATA EVALUATION REPORT

CGA 154'281

Study Type: Combined Chronic Toxicity/Oncogenicity in Rats

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Jennifer E Alexander for Carrie Rabe, Ph.D. Date 5/25/94

Independent Reviewer William L. McFarland for John Liccione, Ph.D. Date 5/25/94

QA/QC Manager ~~Sharon Segal, Ph.D.~~ Carol Macler Ph D Date 5/25/94

Contract Number: 68D10075
Work Assignment Number: 3-09
Clement Number: 35
Project Officer: Caroline Gordon

Guideline 83-5: Combined Chronic Toxicity/Oncogenicity Study

EPA Reviewer: David S. Liem, Ph.D.
Section II, Toxicology Branch II
Health Effects Division

Signature: David S Liem
Date: 9/15/95

EPA Section Head: K. Clark Swentzel
Section II, Toxicology Branch II
Health Effects Division

Signature: K. Clark Swentzel
Date: 9/18/95

DATA EVALUATION REPORT

STUDY TYPE: Combined Chronic Toxicity/Carcinogenicity study in Rats

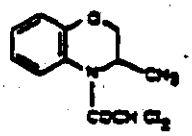
P.C. NO.: 126101

MRID NO.: 428887-04

STUDY NO.: CBG 509/920599 (Sponsor # 891292)

TEST MATERIAL: CGA 154'281 (Benoxacor)

SYNONYM: 4-Dichloroacetyl-3-4-dihydro-3-methyl-2H-1,4-dibenoxazine



SPONSOR: Ciba-Geigy Corporation
Ciba Plant Protection
Greensboro, NC 27419

TESTING FACILITY: Huntingdon Research Centre Ltd.
Huntingdon, Cambridgeshire
England

TITLE OF REPORT: CGA 154'281; Combined Chronic Toxicity/Oncogenicity Study in Rats. Potential Tumorigenic and Toxic Effects in Prolonged Dietary Administration to Rats

AUTHOR: Peter R. Ryle

REPORT ISSUED: April 27, 1993

QUALITY ASSURANCE: A signed Quality Assurance Statement (dated April 26, 1993) and a signed GLP Certification Statement (dated April 27, 1993) were provided. The study was conducted in compliance with OECD guidelines, GLP regulations, EPA FIFRA pesticide guidelines, and Japanese guidelines.

CONCLUSIONS: CGA 154'281 was fed to male and female Crl:CD•BR rats (50/sex/group of the main study plus 20/sex/group of the satellite study) at dietary levels of 0, 10, 50, 500, and 1000 ppm for 2 years. The average daily intakes were 0, 0.4, 2.0, 20.6, and 41 mg/kg/day for males, and 0, 0.6, 2.8, 28.2, and 59 mg/kg/day for females. The following treatment-related effects were observed:

Body weights, body weight gains, food consumption, and feed efficiency were decreased in males and females at 500 ppm (20.6 mg/kg/day for males and 28.2 mg/kg/day for females) and 1000 ppm (41 mg/kg/day and 59 mg/kg/day for males and females, respectively).

At 10 ppm (0.4 mg/kg/day and 0.6 mg/kg/day for males and females, respectively), increases in the incidence of fatty hepatocytes, parafollicular cell hyperplasia in the thyroid, and basophilic cortical tubules in the kidneys were observed in males; in addition, females had congested kidneys and epithelial hyperplasia and hyperkeratosis of the forestomach. Histological lesions at higher doses included liver effects such as cystic bile ducts and centrilobular hypertrophy (with and without vacuolation) (males): changes in the heart pathology (males); and stomach lesions such as raised areas on the epitelial aspect of the forestomach (males and females), nodularity and papillomatous hyperplasia of the limiting ridge (males), and excrescence and papillomatous hyperplasia of the nonglandular stomach (females). Females showed and increased incidence of ovaries without corpora lutea and with follicular cysts.

Peto analysis revealed statistically significant trends for increases in the incidences of squamous cell papillomas in males and squamous cell carcinomas (and carcinomas and/or papillomas) in females in the stomach (epithelial portion of the nonglandular region and/or limiting ridge) at the doses tested under the conditions of this study. However, the incidences of these tumors were not significantly increased by pairwise comparison at any dose.

Based on the above data as presented in the study report the systemic NOEL is determined to be <u>less than</u> 10 ppm (<0.4 and <0.6 mg/kg/day in males and females, respectively).

The systemic LOEL is 10 ppm (0.4 and 0.6 mg/kg/day in males and females, respectively) based on significant increases in the incidences of fatty hepatocytes in males, parafollicular cell hyperplasia in male thyroids, basophilic cortical tubules in male kidneys and congestion in female kidneys.

<u>CORE CLASSIFICATION</u>: The <u>chronic</u> <u>study</u> is classified as <u>core-supplementary</u> because the systemic NOEL could not be determined. However, the carcinogenicity study is classified as <u>core-minimum</u> and satisfies the guideline requirements (§83-2) for a carcinogenicity study in rats.

A. <u>MATERIALS</u>

1. <u>Test Material</u>: CGA 154'281 (Lot# FL881626)

   Description: Brown Crystalline powder

   Purity: 96.4% (information provided by the sponsor)

   Stability: Stable for at least 3 years (as reported by sponsor) expiration date in June, 1993.

   Storage: Room temperature, protected from light

2. <u>Vehicle and/or positive control</u>: Untreated diet

3. Test animals: Species: Rat
Strain: Sprague-Dawley Crl:CD® BR)
Age and weight at study initiation: approximately 6 weeks; males: 152 to 206 g; females: 118 to 166 g
Source: Charles River Laboratories, Inc., Portage, Michigan
Housing: 5 rats/cage
Environmental conditions:

Temperature:	23°C ± 2°C
Humidity:	50% ± 10% 7-8-day
Air changes:	Not specified
Photoperiod:	12 hour light/dark

Acclimation period: 7-8 days

B. STUDY DESIGN:

1. Animals assignment

Animals were assigned randomly to test groups using a stratified body weight method. Animals were uniquely identified using tattoos and ear marks.

Dietary Level (ppm)	Number of Animals			
	Main Study		Satellite Study	
	Males	Females	Males	Females
0	50	50	20	20
10	50	50	20	20
50	50	50	20	20
500	50	50	20	20
1,000	50	50	20	20

Rationale for Dose Selection

Dietary levels of CGA 154'281 for the current study were selected based on the results of a 13-week dietary range-finding study (Project No. 483-243) in which 0, 10, 100, 300, 1,000, and 6,000 ppm were administered to Sprague-Dawley rats. Decreased food consumption, decreased body weight gain, and increased relative liver weight were observed in females at 1,000 ppm. At 6,000 ppm, evidence of hepatotoxicity and nephrotoxicity was reported. This study was not provided for review.

The dietary levels selected for the current study were 0, 10, 50, 500, and 1,000 ppm.

2. Diet preparation and analysis

Test diets were prepared by mixing appropriate amounts of freshly ground test material with the rodent diet (SDS Rat and Mouse No. 1)

to obtain a concentrated premix. Actual dietary concentrations were obtained by diluting the premix with appropriate amounts of rodent chow. Fresh test diets were prepared weekly.

The purity of the test material was verified by the analytical development section of Ciba-Geigy Munchwilen AG both prior to initiation of the study and at study termination. The purity was 95.8% prior to initiation and 96.3% at termination.

Measurement of the homogeneity, stability, and actual concentration of the test material in the diets was conducted by the testing facility using solvent extraction (dichloromethane) followed by high-performance liquid chromatography with spectrophotometric detection. Homogeneity and stability measurements were conducted on samples of diets at 10, 20, and 5,000 ppm prior to initiation of the study. Acceptable homogeneity and stability were achieved. Homogeneity measurements showed that the minimum and maximum concentrations measured at each dietary level were within 15% of each other and the coefficient of variation was <5% at all levels. Stability measurements showed no loss of test material in the diets when stored under animal room conditions for at least 18 days.

Actual levels of test material in the diets were measured at weeks 1, 5, 9, and every 6 weeks thereafter. The average measured concentrations at each test level were as follows:

Nominal Concentration (ppm)	Measured Concentration[a] (ppm)
10	10.1 ± 0.5
50	49.8 ± 1.5
500	488 ± 13
1,000	984 ± 39

[a]Mean ± S.D., calculated by reviewers, data from Addendum 4

3. Animals received food and water ad libitum.

4. Statistical Analyses

Food and water consumption, body weight, organ weight, and clinical pathology data with homogeneous variances (as determined using the Bartlett test) were analyzed as follows: If 75% or more of the data for a particular parameter were equal to the mode, differences from the mode were analyzed using a Fisher's exact test followed by a Mantel's test. For other data, homogeneity of the variance was determined using Bartlett's test, and data with homogeneous variances were analyzed using an analysis of variance. For data with heterogenous variance, a log transformation was attempted prior to

analysis of variance. Following the analysis of variance, differences between treated groups and control groups were determined using a Student's t-test and Williams' test for trends. If the log transformation did not remove heterogeneity, data were analyzed using the Kruskal-Wallis test. A Shirley's test was used to determine between-group differences following the Kruskal-Wallis test.

Organ weight data were analyzed with an analysis of covariance, using body weight at the time of death as the covariate. Mortality was analyzed using a log-rank method. The incidence of nonneoplastic lesions was analyzed using the Fisher's exact test. Neoplastic lesions were analyzed using a Fisher's exact test, or in specific situations, by methods recommended by IARC that take tumor context into consideration (as described by Peto).

5. A signed and dated quality assurance statement was present. A signed and dated GLP statement was present.

C. RESULTS

1. Observations

Animals were observed at least twice daily for toxicity, mortality, and moribundity. In addition, animals were examined weekly for the presence of masses.

Results - No treatment-related effects on survival were observed. Survival to the end of the exposure period (104 weeks) in the main study was as follows:

Dietary Level	Percent Survival	
	Males	Females
0	58	76
10	66	74
50	54	62
500	70	74
1,000	72	62

Data from study CBG 509/920599, Table 2.

No increase in the incidence of overt adverse clinical signs or decrease in the median time of onset were observed. The incidence and location of palpable masses in male and female rats also did not differ significantly between controls and treated animals.

2. Body weight:

Individual body weights were determined weekly for the duration of the study.

Results - Dose-related decreases in body weight and body weight gain were observed in both males and females. At 500 and 1,000 ppm, both males and females had significantly decreased body weights throughout most of the study (Table 1). Male body weights averaged 8% (range, 4-11%) and 10% (range, 6-14%) lower than controls at 500 and 1,000 ppm, respectively. Female body weights averaged 6% (range, 3-11%) and 11% (range, 4-17%) lower than controls at 500 and 1,000 ppm, respectively.

Body weight gain was also significantly decreased in males and females at 500 and 1,000 ppm at most of the intervals presented (Table 2).

3. Food consumption and compound intake

Food consumption values for each cage of animals were determined weekly throughout the study. Feed efficiency was determined during the first 25 weeks of the study at each of the intervals at which body weight gain and food consumption were measured. Test article intake (mg CGA 154'281/kg body weight/day) was calculated weekly during the study using weekly body weight and food consumption data and nominal dietary levels. Water consumption was measured daily over 7-day periods (weeks 12, 25, 51, and 103) for all rats in the satellite group.

Summary data on total food consumption/rat during the first 3, 6, 12, and 24 months of the study showed small (<10%), but statistically significant, decreases in males at 500 and 1,000 ppm at all intervals and in females at 500 and 1,000 ppm during the first year of the study (Table 3). Weekly food consumption data showed statistically significant decreases in both males and females at 500 and 1,000 ppm during the first few weeks of the study, with fairly consistent statistically significant weekly decreases in males at 1,000 ppm and more sporadic decreases thereafter in males at 500 ppm and females at 500 and 1,000 ppm.

Feed efficiency data were presented as food conversion ratios. These data showed decreases in average feed efficiency (increases in the weight of food consumed per unit gain in body weight) in both males and females at 500 and 1,000 ppm (Table 4).

The study author calculated that the mean intake values of CGA 154'281 for male rats receiving diets containing 10, 50, 500, or 1,000 ppm were 0.4, 2.0, 20.6, and 41.0 mg/kg/day, respectively. The intake values for female rats receiving the same dietary concentrations were 0.6, 2.8, 28.2, and 59.0 mg/kg/day, respectively.

Water consumption was significantly decreased (by approximately 20%) in females at 1,000 ppm at weeks 12, 25, and 51 (Table 5).

4. Ophthalmoscopic examination

Eye examinations were conducted on all animals prior to the first exposure to test material, at 52 weeks, and at study termination. The

TABLE 1. Body Weight Data for Rats Ingesting CGA 154'281 in the Diet for up to 2 Years[a,b]

	Body Weight (g) Data (Mean ± S.D.) by Dietary Level (ppm)				
Interval	0	10	50	500	1,000
			Males		
Week 13	542 ± 59	545 ± 61 (101)	540 ± 62 (100)	505 ± 57** (93)	501 ± 59** (92)
Week 26	637 ± 77	644 ± 77 (101)	635 ± 86 (100)	586 ± 75** (92)	583 ± 75** (92)
Week 52	779 ± 104	790 ± 111 (101)	774 ± 119 (99)	725 ± 105** (93)	708 ± 95** (91)
Week 78	884 ± 129	875 ± 142 (99)	821 ± 150 (93)	796 ± 113** (90)	769 ± 101** (87)
Week 104	855 ± 115	870 ± 139 (102)	828 ± 159 (97)	816 ± 153 (95)	781 ± 128* (91)
			Females		
Week 13	264 ± 22	264 ± 24 (100)	263 ± 21 (100)	252 ± 21** (95)	243 ± 18** (92)
Week 26	298 ± 30	300 ± 32 (101)	298 ± 32 (100)	283 ± 25** (95)	271 ± 24** (91)
Week 52	374 ± 52	380 ± 61 (102)	375 ± 56 (100)	350 ± 44** (94)	332 ± 44** (89)
Week 78	443 ± 66	440 ± 80 (99)	452 ± 65 (102)	422 ± 57 (95)	384 ± 57** (87)
Week 104	498 ± 104	483 ± 123 (97)	470 ± 92 (94)	449 ± 88** (90)	419 ± 73** (84)

[a]Data extracted from Study No. CBG 509/920599, Table 4 and Appendix I.
[b]Numbers in parentheses indicate percent control.

* Significantly different from control values, $p \leq 0.05$.
** Significantly different from control values, $p \leq 0.01$.

TABLE 2. Body Weight Gain Data for Rats Ingesting CGA 154'281 in the Diet for up to 2 Years[a,b]

Interval	Body Weight Gain (g) Data (Mean ± S.D.) by Dietary Level (ppm)				
	0	10	50	500	1,000
			Males		
0-13 weeks	359 ± 56	362 ± 57 (101)	356 ± 57 (99)	324 ± 54** (90)	317 ± 57** (88)
0-26 weeks	454 ± 74	461 ± 74 (102)	452 ± 81 (100)	405 ± 72** (89)	399 ± 73** (88)
0-52 weeks	597 ± 102	607 ± 108 (102)	590 ± 114 (99)	543 ± 102** (91)	524 ± 93** (88)
0-104 weeks	672 ± 113	687 ± 136 (102)	648 ± 156 (96)	636 ± 151 (95)	598 ± 127* (89)
			Females		
0-13 weeks	120 ± 17	120 ± 20 (100)	120 ± 17 (100)	109 ± 16** (91)	100 ± 14** (83)
0-26 weeks	154 ± 27	156 ± 29 (101)	155 ± 29 (101)	140 ± 21 (91)	128 ± 20 (83)
0-52 weeks	230 ± 49	236 ± 58 (103)	232 ± 51 (101)	208 ± 41** (90)	188 ± 42** (82)
0-104 weeks	354 ± 103	340 ± 121 (96)	325 ± 91 (92)	306 ± 87* (86)	274 ± 72** (77)

[a]Data extracted from Study No. CBG 509/920599, Table 5.5.
[b]Numbers in parentheses indicate percent control.

* Significantly different from control values, $p \leq 0.05$.
** Significantly different from control values, $p \leq 0.01$.

TABLE 3. Total Food Consumption Data for Rats Ingesting CGA 154'281 in the Diet for up to 2 Years[a,b]

Interval	Total Food Consumption (g/animal) Data (Mean ± S.D.) by Dietary Level (ppm)				
	0	10	50	500	1,000
			Males		
1-13 weeks	2495 ± 130	2542 ± 84 (102)	2516 ± 151 (101)	2363 ± 93** (95)	2302 ± 142** (92)
1-26 weeks	4890 ± 237	4934 ± 159 (101)	4904 ± 291 (100)	4627 ± 183** (95)	4520 ± 257** (92)
1-52 weeks	9718 ± 469	9791 ± 364 (101)	9752 ± 576 (100)	9270 ± 362* (95)	9051 ± 489** (93)
1-104 weeks	19977 ± 1064	19865 ± 696 (99)	19667 ± 1314 (98)	19043 ± 789* (95)	18430 ± 1186** (92)
			Females		
1-13 weeks	1681 ± 76	1712 ± 83 (102)	1693 ± 57 (101)	1580 ± 53** (94)	1583 ± 103** (94)
1-26 weeks	3291 ± 170	3378 ± 140 (103)	3332 ± 103 (101)	3118 ± 94** (95)	3112 ± 189 (95)
1-52 weeks	6616 ± 359	6866 ± 247 (104)	6724 ± 235 (102)	6367 ± 215* (96)	6314 ± 309** (95)
1-104 weeks	14044 ± 664	14718 ± 589 (105)	14136 ± 614 (101)	13590 ± 486 (97)	13560 ± 812 (97)

[a]Data extracted from Study No. CBG 509/920599, Table 5.6.
[b]Numbers in parentheses indicate percent control.

* Significantly different from control values, $p \leq 0.05$.
** Significantly different from control values, $p \leq 0.01$.

TABLE 4. Feed Efficiency Data (g consumption/g body weight gain) for Rats Ingesting CGA 154'281 in the Diet for up to 2 Years[a,b]

Interval	Feed Efficiency Data (Mean) by Dietary Level (ppm)				
	0	10	50	500	1,000
			Males		
weeks 1-25	10.4	10.3 (99)	10.5 (101)	11.0 (106)	11.0 (106)
			Females		
weeks 1-25	20.6	20.7 (100)	20.5 (100)	21.5 (104)	23.4 (114)

[a]Data extracted from Study No. CBG 509/920599, Table 6.
[b]Numbers in parentheses indicate percent control.

TABLE-5. Water Consumption Data (g/animal/week) for Rats Ingesting CGA 154'281 in the Diet for up to 2 Years[a,b]

	Water Consumption Data (Mean ± S.D.) by Dietary Level (ppm)				
Interval	0	10	50	500	1,000
			Males		
Week 12	251 ± 33	243 ± 10 (97)	252 ± 13 (100)	237 ± 18 (94)	244 ± 20 (97)
Week 25	247 ± 50	235 ± 19 (95)	252 ± 16 (102)	237 ± 24 (96)	222 ± 25 (90)
Week 51	238 ± 33	241 ± 14 (101)	252 ± 14 (106)	259 ± 22 (109)	233 ± 12 (98)
Week 103	292 ± 59	277 ± 9 (95)	286 ± 58 (98)	252 ± 13 (86)	279 ± 1 (96)
			Females		
Week 12	197 ± 21	194 ± 16 (98)	186 ± 5 (94)	179 ± 21 (91)	161 ± 17* (82)
Week 25	210 ± 14	210 ± 22 (100)	194 ± 16 (92)	191 ± 17 (91)	164 ± 19** (78)
Week 51	271 ± 30	259 ± 30 (96)	251 ± 38 (93)	239 ± 6 (88)	215 ± 14** (79)
Week 103	372 ± 23	281 ± 19 (76)	284 ± 78 (76)	305 ± 3 (82)	322 ± 77 (87)

[a]Data extracted from Study No. CBG 509/920599, Table 5.9.
[b]Numbers in parentheses indicate percent control.

* Significantly different from control values, $p \leq 0.05$.
** Significantly different from control values, $p \leq 0.01$.

method of examination was not stated, but the report indicated that the pupils were dilated using Tropicamide prior to the examination.

Results - No treatment-related effects on the appearance of the eyes were observed.

5. Clinical Pathology

Hematology and clinical chemistry analyses were performed on 10 rats/sex/dose at 13, 26, 52, 78, and 104 weeks. Satellite animals were primarily used for these analyses. Whenever possible, the same rats were used at each interval. When fewer than 10 rats/sex/dose were available in the satellite groups for these tests, rats from the main study were also used. Blood samples were obtained from the retroorbital sinus of ether-anesthetized rats that had been fasted overnight.

a. Hematology

The parameters marked with an "X" below were examined.

X Hematocrit*
X Hemoglobin*
X Leukocyte count*
X Erythrocyte count*
X Platelet count*
X Reticulocyte count
X RBC morphology
X Leukocyte differential count*
Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Thrombotest

* Recommended by Subdivision F (November 1984) Guidelines

Results - No treatment-related changes in any of the hematology parameters were observed. Statistically significant changes in a few parameters occurred, but these were attributable to a single animal with highly abnormal values, not consistent over time, not dose related, and/or within historical control limits.

b. Clinical chemistry

Blood chemistry analyses were performed for the parameters marked with an "X" below.

Electrolytes
X Calcium*
X Chloride*
X Sodium*
X Phosphorus*
X Potassium*

Enzymes
X Creatine kinase*
X Alkaline phosphatase
X Serum alanine aminotransferase*
X Serum aspartate aminotransferase*
X Gamma glutamyltransferase

Other
X Albumin*
X Total protein*
X Blood creatinine*
X Blood urea nitrogen*
X Total cholesterol*
X Globulin
X Glucose*
X Total bilirubin*
X Uric acid

*Recommended by Subdivision F (November 1984) Guidelines

Results - Treatment-related decreases in total protein and globulin were observed in high-dose males (Table 6). The decreases in total protein and globulin were statistically significant at weeks 13, 52, and 104. Statistically significant changes in these parameters at other doses were not considered to be treatment related because of the absence of a dose response. Although other parameters in treated rats occasionally showed statistically significant differences from controls, these were not considered to be treatment related because of abnormal values among the controls or the absence of a dose response relation.

6. Urinalysis

Urine analyses were performed on 10 rats/sex/dose at 13, 26, 52, 78, and 104 weeks. Satellite animals were primarily used for these analyses. Whenever possible, the same rats were used at each interval. When fewer than 10 rats/sex/dose were available in the satellite groups for these tests, rats from the main study were also used. Urine samples were obtained from metabolism cages in which animals had been housed overnight. Food and water were withheld during the urine collection period.

Urinalysis included the parameters marked with an "X" below.

X Appearance*	X Sediment (microscopic)*	X Bile pigment
X Volume*	X Protein*	X Heme pigment*
X Specific gravity*	X Glucose*	X Urobilinogen
X pH	X Ketones*	X Color

*Recommended by Subdivision F (November 1984) Guidelines

Results - No treatment-related effects were observed.

7. Sacrifice and Pathology

All rats that died, were sacrificed *in extremis*, or were sacrificed as scheduled (using carbon dioxide asphyxiation) received a complete gross examination. The interim sacrifice used 10/sex/dose from the satellite group. Data from remaining satellite animals that died during the study or that were sacrificed at the terminal sacrifice were grouped with data from animals in the main study. Tissues that are marked with an "X" below were examined histologically in all animals. All tissues were preserved in neutral buffered 10% formalin solution (except the eyes which were fixed in Davidson's fixative) prior to paraffin embedding and selection of sections for staining with hematoxylin and eosin. In addition, liver sections (and selected kidney sections) were stained for fat with Oil Red O. Organs that are marked with an "XX" were also weighed at necropsy.

TABLE 6. Selected Clinical Chemistry Data in Rats Ingesting CGA 154'281 in the Diet for up to 2 Years[a,b]

Parameter/Interval	Clinical Chemistry Data (mean ± S.D.) by Dietary Level (ppm)				
	0	10	50	500	1,000
Males					
Total protein (g/dL)					
week 13	7.0 ± 0.2	7.0 ± 0.2 (100)	6.8 ± 0.3 (97)	7.0 ± 0.4 (100)	6.7 ± 0.2* (96)
week 26	7.0 ± 0.3	7.0 ± 0.3 (100)	6.8 ± 0.3 (97)	7.0 ± 0.4 (100)	6.8 ± 0.2 (97)
week 52	7.2 ± 0.3	7.0 ± 0.3 (97)	7.0 ± 0.4 (97)	7.0 ± 0.3 (97)	6.8 ± 0.2* (94)
week 78	7.2 ± 0.4	7.0 ± 0.3 (97)	7.0 ± 0.5 (97)	7.1 ± 0.3 (99)	7.0 ± 0.2 (97)
week 104	7.4 ± 0.5	7.0 ± 0.2* (95)	6.9 ± 0.4* (93)	6.9 ± 0.4* (93)	7.0 ± 0.4* (95)
Globulin (g/dL)					
week 13	4.2 ± 0.2	4.2 ± 0.3 (100)	4.0 ± 0.3 (95)	4.1 ± 0.3 (98)	3.9 ± 0.2** (93)
week 26	4.1 ± 0.2	4.1 ± 0.3 (100)	4.0 ± 0.2 (98)	4.1 ± 0.3 (100)	3.9 ± 0.2 (95)
week 52	4.3 ± 0.3	4.1 ± 0.3 (95)	4.1 ± 0.4 (95)	4.1 ± 0.2 (95)	3.9 ± 0.3* (91)
week 78	4.4 ± 0.5	4.3 ± 0.2 (98)	4.7 ± 0.7 (107)	4.4 ± 0.3 (100)	4.3 ± 0.2 (98)
week 104	4.6 ± 0.6	4.3 ± 0.3 (93)	4.2 ± 0.4* (91)	4.3 ± 0.4* (93)	4.2 ± 0.3* (91)
Females					
Total protein (g/dL)					
week 13	6.8 ± 0.3	7.0 ± 0.6 (103)	6.9 ± 0.4 (101)	6.9 ± 0.3 (101)	6.7 ± 0.4 (99)
week 26	7.0 ± 0.3	7.3 ± 0.4 (104)	7.0 ± 0.3 (100)	7.0 ± 0.4 (100)	7.1 ± 0.6 (101)
week 52	7.4 ± 0.4	7.6 ± 0.4 (103)	7.7 ± 0.3 (104)	7.5 ± 0.4 (101)	7.7 ± 0.4 (104)
week 78	7.2 ± 0.4	7.7 ± 0.3* (107)	7.7 ± 0.3* (107)	7.4 ± 0.4* (103)	7.5 ± 0.4* (104)
week 104	7.1 ± 0.4	7.7 ± 0.5 (108)	7.6 ± 0.6 (107)	7.5 ± 0.5 (106)	7.5 ± 0.6 (106)
Globulin (g/dL)					
week 13	3.7 ± 0.1	3.8 ± 0.3 (103)	3.7 ± 0.2 (100)	3.7 ± 0.2 (100)	3.6 ± 0.3 (97)
week 26	3.7 ± 0.2	3.9 ± 0.3 (105)	3.6 ± 0.2 (97)	3.7 ± 0.2 (100)	3.7 ± 0.4 (100)
week 52	3.8 ± 0.2	3.8 ± 0.3 (100)	4.0 ± 0.2 (105)	3.9 ± 0.3 (103)	4.0 ± 0.2 (105)
week 78	4.0 ± 0.2	4.2 ± 0.3 (105)	4.3 ± 0.2 (108)	4.1 ± 0.4 (103)	4.2 ± 0.4 (105)
week 104	3.9 ± 0.3	4.4 ± 0.4 (113)	4.3 ± 0.4 (110)	4.3 ± 0.6 (110)	4.1 ± 0.4 (105)

[a]Data extracted from Study No. CBG 509/920599, Table 11.
[b]Numbers in parentheses indicate percent control.

* Significantly different from control values, $p \leq 0.05$.
** Significantly different from control values, $p \leq 0.01$.

Digestive System	Cardiovascular/Hematologic	Neurologic
X Pancreas*	X Aorta*	XX Brain*
X Salivary glands*	XX Heart*	X Peripheral nerve* (sciatic nerve)
X Esophagus*	X Bone marrow*	X Spinal cord* (three levels)
X Stomach*	X Lymph nodes*	XX Pituitary*
X Duodenum*	XX Spleen*	X Eyes*
X Jejunum*	X Thymus*	
X Ileum*		
X Cecum*	Urogenital	
X Colon*	X Urinary bladder*	
X Rectum*	XX Kidneys*	Glandular
XX Liver*	XX Testes*	XX Adrenals*
	XX Epididymides*	Lacrimal gland
Respiratory	X Prostate*	X Mammary gland*
X Trachea*	X Vagina*	XX Thyroids*
X Lungs (with mainstem bronchi)*	XX Ovaries*	X Parathyroids*
	X Uterus*	
	X Seminal vesicles*	

Other
X Bone (sternum and femur with joint)*
X Skeletal muscle*
X Tongue
X Skin*
X All gross lesions and masses*

*Recommended by Subdivision F (November 1984) Guidelines

a. Organ weight

No treatment-related changes were observed in absolute organ weights. However, after correcting for body weights, the adjusted liver weights of males at 1,000 ppm were significantly higher (16% greater) than those of controls at the interim sacrifice at week 52 (Table 7).

b. Macroscopic pathology

Treatment-related increases in the incidence of forestomach lesions were observed in male and female rats (Table 8). Increases in the incidence of excrescences were observed in males at 500 and 1,000 ppm and in females at 1,000 ppm (achieving statistical significance in terminal males and females at 1,000 ppm). Raised areas on the epithelial aspect of the forestomach were increased in both males and females at 1,000 ppm (achieving statistical significance in males). In addition, a small increase in the incidence of nodularity of the limiting ridge of the forestomach was observed in males at 1,000 ppm. This effect was not statistically significant.

Treatment-related decreases in the adipose tissue content of rats were also observed. There was an increased total incidence of decreased adiposity in each sex at the top three doses, with statistical significance in males at 50 and 1,000 ppm.

TABLE 7. Liver Weight Data for Rats Ingesting CGA 154'281 in the Diet for up to 2 Years[a,b]

Parameter	Liver Weight (g) Data by Dietary Level (ppm)				
	0	10	50	500	1,000
Males					
Interim					
absolute weight[c]	28.4 ± 5.53	28.6 ± 5.66 (101)	33.2 ± 5.39 (117)	29.1 ± 7.13 (102)	29.9 ± 5.78 (105)
corrected weight[d]	28.1	26.7 (95)	30.2 (107)	31.7 (113)	32.5* (116)
Terminal					
absolute weight[c]	28.7 ± 6.19	28.4 ± 6.04 (99)	27.0 ± 6.72 (94)	27.5 ± 4.51 (96)	28.3 ± 4.85 (99)
corrected weight[d]	28.2	27.7 (98)	27.1 (96)	27.7 (98)	29.3 (104)
Females					
Interim					
absolute weight[c]	16.0 ± 3.18	13.9 ± 1.40 (87)	12.3 ± 2.09 (77)	13.9 ± 1.95 (87)	13.5 ± 2.19 (84)
corrected weight[d]	14.3	13.0 (91)	12.8 (90)	14.8 (103)	14.7 (103)
Terminal					
absolute weight[c]	17.5 ± 4.82	18.6 ± 5.85 (106)	17.3 ± 4.49 (99)	17.2 ± 3.65 (99)	16.5 ± 2.84 (94)
corrected weight[d]	16.1	17.4 (108)	16.6 (103)	17.2 (107)	17.4 (108)

[a]Data extracted from Study No. CBG 509/920599, Table 13.
[b]Numbers in parentheses indicate percent control.
[c]Mean ± S.D.
[d]Values corrected for differences in body weight.

* Significantly different from control value, $p \leq 0.05$.

TABLE 8. Incidence of Macroscopic Findings in Rats Ingesting CGA 154'281 in the Diet for up to 2 Years[a,b]

	Incidence of Macroscopic Findings by Dietary Level (ppm)									
Finding/Interval	0		10		50		500		1,000	
Males										
Adipose tissue										
Minimal										
interim	0/10		0/10		0/10		2/10	(20)	3/10	(30)
intercurrent	0/25		1/21	(5)	7/29*	(24)	3/22	(14)	6/20*	(30)
terminal	2/35	(6)	2/39	(6)	2/31	(6)	2/38	(5)	6/40	(15)
total	2/70	(3)	3/70	(4)	9/70*	(13)	7/70	(10)	15/70**	(21)
Forestomach										
Excrescence(s)										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/25		0/21		0/29		1/22	(5)	0/20	
terminal	1/35	(3)	0/39		0/31		5/38	(13)	12/40**	(30)
total	1/70	(1)	0/70		0/70		6/70	(9)	12/70**	(17)
Raised area(s)-epithelial aspect										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/25		0/21		0/29		0/22		0/20	
terminal	1/35	(3)	0/39		2/31	(6)	2/38	(5)	8/40*	(20)
total	1/70	(1)	0/70		2/70	(3)	2/70	(3)	8/70*	(11)
Limiting ridge nodular										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/25		0/21		0/29		0/22		0/20	
terminal	0/35		0/39		0/31		1/38	(3)	3/40	(8)
total	0/70		0/70		0/70		1/70	(1)	3/70	(4)
Females										
Adipose										
Minimal										
interim	0/10		0/10		0/10		0/10		1/10	(10)
intercurrent	5/16	(31)	5/19	(26)	7/23	(30)	6/15	(40)	9/23	(39)
terminal	1/44	(2)	1/41	(2)	2/37	(5)	4/45	(9)	1/37	(3)
total	4/70	(6)	3/70	(4)	8/70	(11)	8/70	(11)	9/70	(13)
Forestomach										
Excrescence(s)										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/16		0/19		0/23		1/15	(7)	1/23	(4)
terminal	1/44	(2)	1/41	(2)	0/37		2/45	(4)	10/37**	(27)
total	1/70	(1)	1/70	(1)	0/70		3/70	(4)	11/70**	(16)
Raised area(s)-epithelial aspect										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/16		1/19	(5)	0/23		0/15		0/23	
terminal	1/44	(2)	1/41	(2)	0/37		2/45	(4)	4/37	(11)
total	1/70	(1)	2/70	(3)	0/70		2/70	(3)	4/70	(6)
Limiting ridge nodular										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/16		0/19		0/23		0/15		1/23	(4)
terminal	0/44		0/41		1/37	(3)	0/45		0/37	
total	0/70		0/70		1/70	(1)	0/70		1/70	(1)

[a]Data extracted from Study No. CBG 509/920599, Tables 14-16.
[b]Numbers in parentheses indicate percent control.

* Significantly different from control values, $p \leq 0.05$.
** Significantly different from control values, $p \leq 0.01$.

c. Microscopic pathology

1) Nonneoplastic--No treatment-related effects were observed upon microscopic examination of animals sacrificed after 1 year of treatment. The primary treatment-related effects were observed in the stomachs and kidneys of both sexes, and in the livers and thyroids of males at the terminal sacrifice. Other effects were observed in the heart, ovaries, and lungs; these changes appear to reflect a treatment-related increase in age-related lesions (Tables 9a and 9b). Both males and females showed statistically significant increases in the incidence of epithelial hyperplasia and hyperkeratosis of the nonglandular forestomach. This was statistically significant ($p < 0.01$) in males at 1,000 ppm and in females at 50 ppm and above (the effect in females did not increase with dose between 50 and 1,000 ppm). At 1,000 ppm, papillomatous hyperplasia was also observed at the limiting ridge and/or the nonglandular region of the stomach in males and females. This effect achieved statistical significance only in males at the limiting ridge.

Males also showed dose-related increases in the incidence of minimal centrilobular hepatocyte enlargement (either with or without accompanying vacuolation). This effect was statistically significant ($p < 0.01$) at 50 ppm and above. Fat in hepatocytes was statistically increased in males at 10, 50, and 500 ppm, but not at 1000 ppm or in females at any dose. An increase in the incidence of ballooned hepatocytes was also observed in males at 500 and 1,000 ppm at terminal sacrifice. Females did not show similar effects, but a statistically significant increase in the incidence of cystic bile duct was observed in females at 1,000 ppm.

Statistically significant increases in the incidence of minimal-to-moderate myocardial degeneration, fibrosis, vacuolation, and inflammatory cells were also observed in males at 500 and 1,000 ppm. The incidence of this combination of effects at terminal sacrifice was over 90% in males at 500 and 1,000 ppm versus 71% in control males. Males at 1,000 ppm also showed an increased incidence of medial calcification of the blood vessels of the lungs at terminal sacrifice.

Females at the highest dose tested, 1,000 ppm, showed a significant increase in the incidence of ovaries without corpora lutea at terminal sacrifice. In addition, females at 1,000 ppm showed an increase in the incidence of ovaries with follicular cysts among the intercurrent deaths.

Both sexes exhibited kidney effects. In males, there was an elevation of basophilic cortical tubules at all treatment levels, with significance at 50 ppm. In females, kidney congestion was increased at all doses, with significance at 10 and 50 ppm. In addition, males showed a significant

KEY TO TABLE 9

[a]Data extracted from Study No. CBG 509/920599, Tables 19 and 22.
[b]Numbers in parentheses indicate percent incidence.
[c]Severity ratings were not given for each afflicted animal at this dose for this parameter; in occasional rats, lesions were rated only as present.

I = incidence
AS = average severity (where 0.5 = trace, 1 = minimal, 2 = moderate, and 3 = marked)
P = present

* Significantly different from control values, $p \leq 0.05$.
** Significantly different from control values, $p \leq 0.01$.

TABLE 9a. Incidence and Severity of Nonneoplastic Lesions in Male Rats Ingesting CGA 154'281 in the Diet for up to 2 Years[a,b]

Lesion/Interval	0 ppm		10 ppm		50 ppm		500 ppm		1,000 ppm	
	I	AS	I	AS	I	AS	I	AS	I	AS
Stomach										
Epithelial hyperplasia and hyperkeratosis non-glandular region										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	10/25 (40)	1.7	2/21 (10)	1.5	2/29 (7)	1.5	6/22 (27)	1.5	2/20 (10)	1.0
terminal	10/35 (29)	1.0	9/39 (23)	1.2	13/31 (42)	1.2	16/38 (42)	1.2	33/40**(83)	1.2[c]
total	20/70 (29)	1.4	11/70 (16)	1.3	15/70 (21)	1.3	22/70 (40)	1.3	35/70**(50)	1.2
Papillomatous hyperplasia non-glandular region										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	2/25 (8)	1.0	2/21 (10)	1.5	2/29 (7)	1.5	0/22		1/20 (5)	2.0
terminal	1/35 (3)	2.0	0/39		1/31 (3)	1.0	3/38 (8)	1.0[c]	6/40 (15)	1.2
total	3/70 (4)	1.3	2/70 (3)	1.5	3/70 (4)	1.3	3/70 (4)	1.0	7/70 (10)	1.3
at the limiting ridge										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/25		0/21		0/29		0/22		1/20 (5)	P
terminal	0/35		1/39 (3)	P	1/31 (3)	P	3/38 (8)	P	6/40* (15)	P
total	0/70		1/70 (1)	P	1/71 (1)	P	3/70 (4)	P	7/70**(10)	P
Liver										
Centrilobular enlargement and vacuolation of hepatocytes										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/25		0/21		3/29 (10)	1.0	1/22 (5)	1.0	4/20* (20)	1.0
terminal	0/35		3/39 (8)	1.0	7/31**(23)	1.0	11/38**(29)	1.0	20/40**(50)	1.1
total	0/70		3/70 (4)	1.0	10/70**(14)	1.0	12/70**(17)	1.0	24/70**(34)	1.0
Centrilobular hepatocyte enlargement										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/25		0/21		3/29 (10)	1.0	4/22* (18)	1.0	2/20 (10)	1.0
terminal	1/35 (3)	1.0	5/39 (13)	1.0	6/31* (19)	1.0	12/38**(32)	1.2	14/40**(35)	1.2
total	1/70 (1)	1.0	5/70 (7)	1.0	9/70**(13)	1.0	16/70**(23)	1.1	16/70**(23)	1.2
Ballooned cells										
interim	0/10		0/10		1/10 (10)	P	0/10		0/10	
intercurrent	8/25 (32)	P	5/21 (24)	P	7/29 (24)	P	5/22 (23)	P	2/20 (10)	P
terminal	15/35 (43)	P	14/39 (36)	P	18/31 (58)	P	25/38* (66)	P	29/40**(73)	P
total	23/70 (33)	P	19/70 (27)	P	26/70 (37)	P	30/70 (43)	P	31/70 (44)	P
Cystic bile duct with fibrosis										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/25		0/21		0/29		0/22		0/20	
terminal	0/35		0/39		0/31		0/38		1/40 (3)	P
total	0/70		0/70		0/70		0/70		1/70 (1)	P
Fat in hepatocytes										
interim	0/10		2/10 (20)	0.8	2/10 (20)	0.8	0/10		2/10 (20)	0.5
intercurrent	2/25 (8)	1.5	7/21*(33)	0.9	5/29 (17)	1.6	8/22* (36)	1.8	3/20 (15)	1.2
terminal	3/35 (9)	1.3	6/39 (15)	0.7	8/31 (26)	1.3	11/38 (29)	1.1	0/40	
total	5/70 (7)	1.4	15/70*(21)	0.8	15/70* (21	1.2	19/70**(27)	1.5	5/70 (7)	0.9

TABLE 9a (Continued)

Lesion/Interval	0 ppm		10 ppm		50 ppm		500 ppm		1,000 ppm	
	I	AS	I	AS	I	AS	I	AS	I	AS
Heart										
Myocardial degeneration, fibrosis, vacuolation, and inflammatory cells										
interim	1/10 (10)	1.0	0/10		0/10		0/10		1/10 (10)	1.0
intercurrent	11/25 (44)	1.2	15/21 (71)	1.3	17/29 (59)	1.4	14/22 (64)	1.6	10/20 (50)	1.2
terminal	25/35 (71)	1.2	32/39 (82)	1.3	28/31 (90)	1.3	35/38* (92)	1.3	37/40* (93)	1.2
total	37/70 (53)	1.2	47/70 (67)	1.3	45/70 (64)	1.3	49/70* (70)	1.3	48/70* (69)	1.2
Mast cells										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	3/25 (9)	P	1/21 (5)	P	2/29 (7)	P	6/22 (27)	P	1/20 (5)	P
terminal	9/35 (26)	P	17/39 (44)	P	7/31 (23)	P	13/38 (34)	P	11/40 (28)	P
total	12/70 (17)	P	18/70 (26)	P	9/70 (13)	P	19/70 (27)	P	12/70 (17)	P
Lungs										
Medial calcification of blood vessel										
interim	6/10 (60)	P	7/10 (70)	P	8/10 (80)	P	5/10 (50)	P	6/10 (60)	P
intercurrent	19/25 (76)	P	16/21 (76)	P	24/29 (83)	P	15/22 (68)	P	16/20 (80)	P
terminal	25/35 (71)	P	32/39 (82)	P	28/31 (90)	P	31/38 (82)	P	36/40* (90)	P
total	50/70 (71)	P	55/70 (79)	P	60/70* (86)	P	51/70 (73)	P	58/70 (83)	P
Spleen										
Hemosiderosis										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	3/25 (12)	1.0	6/21 (29)	1.2	8/29 (28)	1.4	7/22 (32)	1.3	8/20* (40)	1.5
terminal	1/35 (3)	1.0	0/39		2/31 (6)	1.0	1/38 (3)	1.0	3/40 (8)	1.0
total	4/70 (6)	1.0	6/70 (9)	1.2	10/70 (14)	1.2	8/70 (11)	1.2	11/70* (16)	1.3
Kidneys										
Basophilic cortical tubules										
interim	1/10 (10)	0.5	0/10		1/10 (10)	1.0	2/10 (20)	1.0	3/10 (30)	0.8
intercurrent	0/25		4/21* (19)	1.1	8/29** (28)	0.9	1/22 (5)	1.0	2/20 (10)	1.7
terminal	6/35 (17)	0.8	10/39 (26)	0.8	9/31 (29)	0.8	11/38 (29)	0.9	5/40 (13)	0.8
total	7/70 (10)	0.7	14/70 (20)	1.0	18/70* (26)	0.9	14/70 (20)	1.0	10/70 (14)	1.1
Congestion										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	12/25 (48)	P	4/21 (19)	P	7/29 (24)	P	5/22 (23)	P	3/20 (15)	P
terminal	17/35 (49)	P	20/39 (51)	P	16/31 (52)	P	18/38 (47)	P	20/40 (50)	P
total	29/70 (41)	P	24/70 (34)	P	23/70 (33)	P	23/70 (33)	P	23/70 (33)	P
Thyroids										
Parafollicular cell hyperplasia										
interim	1/10 (10)	P	0/10		0/10		0/10		0/10	
intercurrent	0/25		5/21* (24)	P	5/29* (17)	P	1/22 (5)	P	3/20 (15)	P
terminal	1/35 (3)	P	9/39* (23)	P	6/31* (19)	P	5/38 (13)	P	4/40 (10)	P
total	2/70 (3)	P	14/70** (20)	P	11/70** (16)	P	6/70 (9)	P	7/70 (10)	P
Adrenals										
Medullary hyperplasia										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	2/25 (8)	P	2/21 (10)	P	4/29 (14)	P	4/22 (18)	P	5/20 (25)	P
terminal	5/35 (14)	P	3/39 (8)	P	7/31 (23)	P	7/38 (18)	P	10/40 (25)	P
total	7/70 (10)	P	5/70 (7)	P	11/70 (16)	P	11/70 (16)	P	15/70 (21)	P

TABLE 9b. Incidence and Severity of Nonneoplastic Lesions in Female Rats Ingesting CGA 154'281 in the Diet for up to 2 Years[a,b]

Lesion/Interval	0 ppm		10 ppm		50 ppm		500 ppm		1,000 ppm	
	I	AS	I	AS	I	AS	I	AS	I	AS
Stomach										
Epithelial hyperplasia and hyperkeratosis non-glandular region										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	1/16 (6)	2.0	6/19 (32)	1.2	3/23 (13)	1.0	0/15		3/23 (13)	1.0
terminal	12/44 (27)	1.1	16/41 (39)	1.3	28/37**(76)	1.0	29/45**(64)	1.1	28/37**(76)	1.1
total	13/70 (19)	1.2	22/70 (31)	1.2	31/70**(44)	1.0	29/70**(41)	1.1	31/70**(44)	1.1
Papillomatous hyperplasia non-glandular region										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/16		2/19 (11)	1.0	0/23		1/15 (7)	1.0	0/23	
terminal	1/44 (2)	1.0	2/41 (5)	1.0	1/37 (3)	1.0	3/45 (7)	1.0	6/37* (16)	1.2[c]
total	1/70 (1)	1.0	4/70 (6)	1.0	1/70 (1)	1.0	4/70 (6)	1.0	6/70 (9)	1.2
at the limiting ridge										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/16		0/19		0/23		0/15		1/23 (4)	P
terminal	0/44		1/41 (2)	P	0/37		1/45 (2)	P	2/37 (5)	P
total	0/70		1/70 (1)	P	0/70		1/70 (1)	P	3/70 (4)	P
Liver										
Centrilobular enlargement and vacuolation of hepatocytes										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/16		0/19		0/23		0/15		0/23	
terminal	0/44		0/41		0/37		0/45		0/37	
total	0/70		0/70		0/70		0/70		0/70	
Centrilobular hepatocyte enlargement										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/16		1/19 (5)	1.0	0/23		0/15		0/23	
terminal	0/44		0/41		0/37		0/45		0/37	
total	0/70		1/70 (1)	1.0	0/70		0/70		0/70	
Ballooned cells										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/16		1/19 (5)	P	1/23 (4)	P	0/15		1/23 (4)	P
terminal	2/44 (5)	P	2/41 (5)	P	3/37 (8)	P	1/45 (2)	P	1/37 (3)	P
total	2/70 (3)	P	3/70 (4)	P	4/70 (6)	P	1/70 (1)	P	2/70 (3)	P
Cystic bile duct										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/16		0/19		0/23		0/15		1/23 (4)	P
terminal	0/44		0/41		0/37		1/45 (2)	P	4/37* (11)	P
total	0/70		0/70		0/70		1/70 (1)	P	5/70* (7)	P
Fat in hepatocytes										
interim	5/10 (50)	0.5	5/10 (50)	0.5	2/10 (20)	0.5	1/10 (10)	0.5	2/10 (20)	0.5
intercurrent	3/16 (19)	1.7	1/19 (5)	2.0	3/23 (13)	1.8	2/15 (13)	1.3	0/23	
terminal	2/44 (5)	0.5	2/41 (5)	0.8	1/37 (3)	1.0	1/45 (2)	1.0	1/37 (3)	0.5
total	10/70 (14)	0.9	8/70 (11)	1.1	6/70 (9)	1.1	4/70 (6)	0.9	3/70 (4)	0.5

TABLE 9b (Continued)

Lesion/Interval	0 ppm		10 ppm		50 ppm		500 ppm		1,000 ppm	
	I	AS	I	AS	I	AS	I	AS	I	AS
Heart										
Myocardial degeneration, fibrosis, vacuolation, and inflammatory cells										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	3/16 (19)	0.8	1/19 (5)	1.0	9/23 (39)	0.9	4/15 (27)	1.0	4/23 (17)	0.9
terminal	20/44 (45)	1.1	19/41 (46)	1.1	25/37* (68)	1.0	25/45 (56)	1.0	21/37 (57)	1.0
total	23/70 (33)	1.0	21/70 (30)	1.0	34/70* (49)	1.0	29/70 (41)	1.0	25/70 (36)	1.0
Mast cells										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/16		2/19 (11)	P	0/23		1/15 (7)	P	2/23 (9)	P
terminal	5/44 (11)	P	16/41** (39)	P	4/37 (11)	P	8/45 (18)	P	8/37 (22)	P
total	5/70 (7)	P	18/70** (26)	P	4/70 (6)	P	9/70 (13)	P	10/70 (14)	P
Lungs										
Medial calcification of blood vessel										
interim	4/10 (40)	P	6/10 (60)	P	4/10 (40)	P	5/10 (50)	P	5/10 (50)	P
intercurrent	13/16 (81)	P	12/19 (63)	P	16/23 (70)	P	11/15 (73)	P	17/23 (74)	P
terminal	33/44 (75)	P	29/41 (71)	P	27/37 (73)	P	34/45 (76)	P	28/37 (76)	P
total	50/70 (71)	P	47/70 (67)	P	47/70 (67)	P	50/70 (71)	P	50/70 (71)	P
Spleen										
Hemosiderosis										
interim	7/10 (70)	2.0	5/10 (50)	1.2	4/10 (40)	1.5	10/10 (100)	1.2	7.10 (70)	2.0
intercurrent	12/16 (75)	1.1	19/19* (100)	1.9	19/23 (83)	1.9	12/15 (80)	1.9	19/23 (83)	1.9
terminal	35/44 (80)	1.5	33/41 (80)	1.6	34/37 (92)	1.5	34/45 (76)	1.4	33/37 (89)	1.5
total	54/70 (77)	1.5	57/70 (81)	1.6	57/70 (81)	1.6	56/70 (80)	1.5	59/70 (84)	1.8
Kidneys										
Congestion										
interim	0/10		0/10		0/10		0/10		0/10	
intercurrent	0/16		1/19 (5)	P	2/23 (9)	P	1/15 (7)	P	3/23 (13)	P
terminal	15/44 (34)	P	31/41** (76)	P	23/37* (62)	P	20/45 (44)	P	19/37 (51)	P
total	15/70 (21)	P	32/70** (46)	P	25/70** (36)	P	21/70 (30)	P	22/70 (31)	P
Thyroids										
Parafollicular cell hyperplasia										
interim	1/10 (10)	P	1/10 (10)	P	3/10 (30)	P	0/10		1/10 (10)	P
intercurrent	2/16 (13)	P	2/19 (11)	P	1/23 (4)	P	3/15 (20)	P	1/23 (4)	P
terminal	5/44 (11)	P	6/41 (15)	P	4/37 (11)	P	3/45 (7)	P	2/37 (5)	P
total	8/70 (11)	P	9/70 (13)	P	8/70 (11)	P	6/70 (9)	P	4/70 (6)	P
Ovaries										
Follicular cysts										
interim	0/10		0/10		2/10 (20)	P	3/10 (30)	P	1/10 (10)	P
intercurrent	0/16		1/19 (5)	P	3/23 (13)	P	2/15 (13)	P	7/23* (30)	P
terminal	10/44 (23)	P	5/41 (12)	P	8/37 (22)	P	11/45 (24)	P	12/37 (32)	P
total	10/70 (14)	P	6/70 (9)	P	13/70 (19)	P	16/70 (23)	P	20/70* (29)	P
Corpora lutea absent										
interim	9/10 (90)		5/10 (50)		2/10 (20)		6/10 (60)		6/10 (60)	
intercurrent	9/16 (56)		9/19 (47)		9/23 (39)		4/15 (27)		9/23 (39)	
terminal	6/44 (14)		12/41 (29)		9/37 (24)		6/45 (13)		13/37* (35)	
total	24/70 (34)		26/70 (37)		20/70 (29)		16/70 (23)		28/70 (40)	

increase in splenic hemosiderosis at the top dose, and parafollicular cell hyperplasia in male thyroids was significantly increased at 10 and 50 ppm, but not at higher doses.

2) Neoplastic--Peto analysis revealed significant dose-related trends in the incidence of squamous cell papillomas of the stomach (nonglandular epithelium and/or limiting ridge) in males. A similar analysis in females revealed significant positive trends for the incidence of squamous cell carcinomas and carcinomas and/or papillomas of the epithelial portion of the nonglandular stomach (Table 10). Historical control data from 18 studies conducted in the test laboratory showed a very low spontaneous incidence of such tumors (0/60-2/50 in males and 0/60-1/52 in females).

Slight increases in the incidence of benign hepatocellular tumors were observed in treated males and females; however, the incidence of these tumors was not considered to be treatment-related because the incidence was comparable to that in historical controls and no statistically significant increases in incidence or trends were observed.

D. DISCUSSION

The design and conduct of this study were complete and adequate, and the summary table data were supported by the individual animal data. The data were well reported, and the statistics used were appropriate.

General evidence of toxicity was apparent in both males and females at 500 and 1,000 ppm as small decreases in body weight, body weight gain, food consumption, and feed efficiency. In males at 1,000 ppm, there was an accompanying increase in the incidence of decreased adipose tissue at necropsy. The major target organs in rats ingesting CGA 154'281 for up to 2 years were the forestomach and liver. Effects in the stomach were not apparent after 1 year of exposure but were observed in multiple animals at terminal sacrifice. Effects observed macroscopically in the forestomach of males and/or females included increases in the incidences of excrescences, raised areas on the epithelial aspect, and nodularity at the limiting ridge. At the microscopic level, these changes corresponded to increases in the incidences of epithelial hyperplasia and hyperkeratosis of the nonglandular stomach and papillomatous hyperplasia of the limiting ridge and/or nonglandular stomach. These changes were primarily observed in high-dose animals; however, females at doses as low as 50 ppm had statistically significant increases in epithelial hyperplasia and hyperkeratosis of the nonglandular forestomach, and males at 500 ppm had a nonsignificant increase in the incidence of macroscopically observed excrescences. Although no statistically significant increase was observed in the incidence of forestomach tumors at any given dose level, Peto analysis revealed statistically significant increasing trends for squamous cell papillomas (males), carcinomas (females), and papillomas and/or carcinomas (females) in the nonglandular and/or limiting ridge of the stomach.

TABLE 10. Incidence of Neoplastic Findings in Rats Ingesting CGA 154'281 in the Diet for up to 2 Years[a,b]

Finding/Interval	Incidence of Neoplastic Findings by Dietary Level (ppm) 0	10	50	500	1,000
Males					
Squamous cell papillomas					
-non-glandular region					
interim	0/10	0/10	0/10	0/10	0/10
intercurrent	0/25	0/21	0/29	0/22	0/20
terminal	0/35	0/39	0/31	0/38	3/40 (8)
total	0/70	0/70	0/70	0/70	3/70 (4)
-at the limiting ridge					
interim	0/10	0/10	0/10	0/10	0/10
intercurrent	0/25	0/21	0/29	0/22	0/20
terminal	0/35	0/39	0/31	0/38	2/40 (5)
total	0/70	0/70	0/70	0/70	2/70 (3)
-non-glandular region and/or at the limiting ridge[c]					
interim	0/10	0/10	0/10	0/10	0/10
intercurrent	0/25	0/21	0/29	0/22	0/20
terminal	0/35	0/39	0/31	0/38	4/40 (10)
total	0/70	0/70	0/70	0/70	4/70 (6)
Squamous cell carcinomas					
-non-glandular region					
interim	0/10	0/10	0/10	0/10	0/10
intercurrent	0/25	0/21	0/29	0/22	0/20
terminal	0/35	0/39	0/31	0/38	0/40
total	0/70	0/70	0/70	0/70	0/70
Females					
Squamous cell papillomas					
-non-glandular region					
interim	0/10	0/10	0/10	0/10	0/10
intercurrent	0/16	0/19	0/23	0/15	0/23
terminal	1/44 (2)	0/41	0/37	1/45 (2)	4/37 (11)
total	1/70 (1)	0/70	0/70	1/70 (1)	4/70 (6)
-at the limiting ridge					
interim	0/10	0/10	0/10	0/10	0/10
intercurrent	0/16	0/19	0/23	0/15	0/23
terminal	0/44	0/41	0/37	0/45	0/37
total	0/70	0/70	0/70	0/70	0/70
-non-glandular region and/or at the limiting ridge					
interim	0/10	0/10	0/10	0/10	0/10
intercurrent	0/16	0/19	0/23	0/15	0/23
terminal	1/44 (2)	0/41	0/37	1/45 (2)	4/37 (11)
total	1/70 (1)	0/70	0/70	1/70 (1)	4/70 (6)
Squamous cell carcinomas					
-non-glandular region					
interim	0/10	0/10	0/10	0/10	0/10
intercurrent	0/16	0/19	0/23	0/15	0/23
terminal	0/44	0/41	0/37	0/45	1/37 (3)
total	0/70	0/70	0/70	0/70	1/70 (1)

[a]Data extracted from Study No. CBG 509/920599, Table 21 and Appendix 9.
[b]Numbers in parentheses indicate percent incidence.
[c]One of the males was found to have both a squamous cell papilloma at the non-glandular region and a squamous cell papilloma at the limiting ridge.

Hepatic effects were observed predominantly in males. At doses as low as 50 ppm, males exhibited centrilobular hepatocyte enlargement both with and without accompanying vacuolation. Fat in hepatocytes was significantly increased at all treatment levels except the top dose. At 500 ppm and above, an increase in the incidence of ballooned hepatocytes was evident. At the highest dose tested, males had decreased serum total protein and globulin (probably associated with the hepatotoxicity). A transient (seen only at the interim sacrifice) increase in liver weights of males at 1,000 ppm (after correction for body weight differences) was also observed. Females at 1,000 ppm exhibited an increase in the incidence of cystic bile duct.

Additional treatment-related lesions included increases in myocardial degeneration, fibrosis, vacuolation, and infiltration with inflammatory cells in males at 500 and 1,000 ppm, medial calcification of the blood vessels of the lungs in males at 1,000 ppm, and increases in the incidence of ovaries without corpora lutea or with follicular cysts in females at 1,000 ppm. Other multiple-dose elevations included male splenic hemosiderosis (significant at 1000 ppm), and parafollicular cell hyperplasia in male thyroids (significant at the top two doses). In both sexes, kidney effects were observed. These effects included basophilic cortical tubules in males and congestion in females, and were significant in each sex at 10 and 50 ppm. Non-neoplastic toxicological effects were discussed with L. Brennecke, pathologist.

These results are consistent with those observed in an oncogenicity study with CGA 154'281 in mice (MRID 428887-02) which showed no effects at 30 ppm and increases in the incidence of forestomach excrescences (males and females) at 600 ppm. At 1,200 ppm, the mice exhibited decreased body weight (males), body weight gain (males), and adipose tissue (males) and increased absolute liver weight (males and females), liver enlargement (females), parenchymal inflammation of the liver (females), splenic hemosiderosis (females), ovaries with hemorrhagic cysts (females), squamous cell papillomas of the nonglandular portion of the stomach (males and females), papillomatous and epithelial hyperplasia of the nonglandular portion of the the stomach (males), and an increasing trend for squamous cell carcinomas of the nonglandular portion of the stomach (males).

Results of the chronic dog study (MRID 428887-01) indicate that adjusted liver and kidney weights were increased in males and females, and lipofuscin deposition in the kidneys of males and females was increased. In addition, red cell parameters in two high-dose males were depressed and accompanied by increased spleen weights

In the current study, the NOEL for systemic toxicity was less than 10 ppm. The LOEL is 10 ppm, based on significant increases (accompanied by multiple-dose elevations) in fatty hepatocytes (males), thyroid parafollicular cell hyperplasia (males), and kidney congestion (females). At all higher doses, the incidence of centrilobular hypertrophy (with and without vacuolation) was significantly increased in males. In addition, epithelial hyperplasia and hyperkeratosis of the forestomach was significantly increased in females at 50, 500, and 1000 ppm. In addition, although no statistically significant increase in the incidence of tumors was observed at any dose level by pairwise comparison, Peto analysis

revealed statistically significant increasing trends for squamous cell papillomas (males), carcinomas (females), and papillomas and/or carcinomas (females in the nonglandular forestomach.

The chronic study is classified as core-supplementary because the systemic NOEL could not be determined. However, the carcinogenicity study is classified as core-minimum and satisfies the guideline requirements (§83-2) for a carcinogenicity study in rats.

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